

New joint ventures in Europe

Next week's meeting of European science ministers at Paris is unlikely to accomplish much, but at least it makes a start on the improvement of science collaboration. Where next?

THE Council of Europe can hardly be accused of being bossy. Its faults are rather those of ineffectiveness. The council (which, with twenty-one members, covers a wider swathe in Europe than, say, the European Communities) is best known as the source of international conventions which have the force of law in Europe when ratified by member governments. (The human rights convention works well, the convention on laboratory animals has been five years in negotiation and is still not complete.) The council also stages large inter-governmental conferences of monumental banality such as that labelled "Education 2000" at Strasbourg (the council's home base) last November and that on communications and society. So how does such an organization come to be staging an important meeting of European ministers responsible for research at Paris this weekend? And can it be trusted with the responsibility?

The oddest feature of the meeting is not the agenda, which is excessively modest, but that there is one of any kind. The origin of next week's meeting goes back to the stirring speech to the council's parliamentary assembly by M. François Mitterrand, the President of France, on what has become the characteristic French theme in Europe (and in France) — that Europe's economic future rests on the better use of science and technology. What Charlemagne once did by conquest, to create economic and political power out of unity, M. Mitterrand would accomplish by the coordination of European research and development. M. Mitterrand can congratulate himself that he has been listened to so well. And if the agenda for next weekend is only modest, there is always a chance that it will lead to better things.

Objectives

The omens are encouraging. Even governments such as the British, whose hostility to much of the council's work stems not so much from insularity as from the wish not to be distracted from preoccupations of its own choosing, seems willing to go along with what is proposed. Two objectives dominate the agenda — the improvement of the networks linking research people in Europe, and providing encouragement to the mobility of scientists. If all turns out as planned, the chief beneficiary of the conference next week will be the European Science Foundation (also Strasbourg-based), which will be urged to encourage the development of research networks on a European basis (but told to raise the money for that from its members, mostly European research councils). This is a timely recognition of the foundation's success, during the past decade, in creating mechanisms of collaboration in fields as different as neurophysiology and the uses of synchrotron radiation (and the development of sources thereof). The other theme, mobility, is necessarily more nebulous. Everybody recognizes that the free movement of research people within Europe would be good for science as well as for the economies of Europe, but there are serious barriers to mobility, deriving from chauvinism and pettifogging administration, to be overcome. The hope seems to be that the Council of Europe and the European Commission can jointly embark on the removal of some of these obstacles once the weekend is over.

That, for those who attend, is the applause. The other side of the coin, of which they should be made aware, is that the agenda on which they will be brooding is about as modest as any that

could have been devised. In the spirit of M. Mitterrand's rhetoric, and no doubt against the background of the many high-sounding things that will be said in Paris, the agreements that will be reached are bound to seem like an anticlimax. Charlemagne made the Rhine into the axis of economic power. But the science ministers — on the same metaphor — will be invited next weekend merely to test the salinity of the river at a few selected points. European politics being what they are, there may be no other choice. But the ministers (and M. Mitterrand) should acknowledge how wide a gulf separates their ambitions and what is likely to be achieved.

The problem

The European dilemma is simply stated. Many Europeans think it prudent to opt out of the ambition of making Europe into an integrated economic power, counting institutions such as the European Communities and the Council of Europe as institutions whose value must continually be reassessed. Even the others, those who hold that European integration in some form may be more than a pragmatic benefit, are divided into separate groups — those who hold that the forging of economic strength in Europe must be an evolutionary process, one small thing leading to another hardly any bigger, and those (numerically fewer) who would prefer to take the bull by its constitutional horns (which is what the signatories of the Treaty of Rome thought they were doing thirty years ago). Rhetoric apart, this weekend's meeting is squarely but limply in the evolutionary tradition.

The present difficulty in Europe is that both approaches are likely to be ineffectual. Softly-softly is too slow. But even constitutional change is frustrated by what are known in the European Communities as "non-tariff barriers to trade". People pay lip-service to the notion that it would be good for Europe if the most efficient manufacturers of, say, mousetraps enjoyed the best sales but they are also willing to bend the rules so as to make sure that only nationally manufactured mousetraps really succeed. In this spirit, one of the European Communities' collaborative projects in technology, the Esprit computer development programme will be a simple and a shocking waste of quite a lot of money if its outcome has to contend with the long-standing chauvinism of European governments towards the products of high technology. Even the modest proposals being discussed in Paris at the weekend will be frustrated if approval for the greater mobility of technical people is alloyed with too much concern that public research funds should always be spent by those who have contributed towards them as taxpayers or that public laboratories should employ only local nationals.

President Mitterrand's ambition will never be achieved if Europe insists on putting the cart before the horse in ways like that. The logic is impeccable. Numerically, even the European Communities are large and rich enough to be an economic unit comparable in size with any others in the world, while the populations concerned are amongst the most literate and skilled that are anywhere to be found. Moreover, Europe is blessed with modern social and economic institutions — universities, for example, or commercial companies and investment institutions. But as things are, the dream is belied by the reality. European governments are these days being repeatedly reminded of how they are slipping in their performance. The British Government,



for example, after four years of energetic sponsorship of what it calls information technology, should have been shattered two weeks ago to have been told by a committee of the National Economic Development Office under Professor John Ashworth that this same period has seen a steady deterioration in the balance of external trade in equipment such as computing machinery. The difficulty is that a home-grown industry only poorly supported by research and development, and facilities for education and training, is being exhorted to compete across the board with stronger organizations overseas.

What next weekend's meeting should also recognize is that there is a sharp difference of timing between the urgency of these problems of industrial competitiveness and the time-scale implied by its own agenda. Stronger networks in research are, to be sure, desirable, but will they help with the problems of industrial survival cropping up all over Europe? The simple answer is that they will not. The Council of Europe is, in any case, out of its depth in fields such as this. But the recognition that opportunities are being lost with every year should at least give those assembling in Paris a sense that if their weekend is to be usefully spent they had better look further ahead.

What might the council achieve? New actors can always hope to breathe new life into old parts especially because, since the council's constituency extends between the European Communities, evolutionary and not constitutional approaches are unavoidable. One worthwhile ambition, however, would be to embark on the encouragement of integration in higher education, both in teaching and academic research. Why not seek to engineer the return to the fourteenth century, when it was commonplace that both the students and teachers of European universities were recruited from the whole continent? Why not even go one step further and acknowledge that if the objective is true European scholarship, it will at some stage be necessary that public spending on the support of scholarship, even by national governments, should be channelled always to teachers and people most likely to make use of it? It is unreasonable to expect all scholastic steps in this direction to be taken quickly, but it is illogical not to prepare for it while otherwise behaving as if President Mitterrand's speeches should be taken seriously. □

Election irrelevant

Something must be done about the federal deficit, whatever the outcome of the US election.

WILL interest rates go up or down after the November presidential election in the United States? And will the dollar, now (with the yen) the strongest of international currencies, then lose some of its recent shine? Voters in the United States should know that these questions (together with the administration's policy on arms control) are elsewhere more absorbing and more important than the intricacies of the candidates' relationships with the Inland Revenue Service. Future prosperity in the rest of the world, industrialized and poverty-stricken alike, will be determined by the economic fall-out of the election. The crucial question is how the paradox of US economic policy is eventually to be unscrambled.

The essence of the paradox is now boringly familiar. The federal government has failed to balance its budget to the tune of \$200,000 million a year, and seems likely to continue in that way. Only last weekend, President Reagan told an election audience that he had no special plans to act on the deficit after the election, saying that continued growth might automatically redress the balance by increasing tax revenues. The result is that the US Treasury has had to borrow from the money markets to finance its research, to pay salaries to those who work in government laboratories, for example. High interest rates are not, however, necessarily a precondition for successful borrowing, but they are the only way of financing a huge deficit while keeping inflation in check. Everybody agrees about that.

It is also agreed that US policy has been successful within its lights. Inflation has indeed been kept at bay, which is why not

only US citizens but people and institutions from overseas are eager to lend to the US Government. So money has been rushing into the United States. The penalty, a consequence of short-term confidence in the dollar, is that goods and services in the United States have become relatively less competitive, so that the trade balance has deteriorated. This year, the United States will not be paying in the usual way for \$85,000 million of the goods and services it will be importing, a deficit comparable in scale with the surpluses earned by the oil exporters in the 1970s. That, the United States demonstrated at the end of the 1960s, when part of the cost of the Vietnam war was paid for with paper money printed on the Eurodollar market, which turned out to be a way of exporting inflation. Since then, however, other governments have learned a thing or two, but their only defence against the resurgence of inflation is to keep their own interest rates at a level determined by those in the United States. The paradox is that those who lend money internationally do not usually do so with confidence when a creditor country is in the red both on its fiscal accounts and its balance of trade. Circumstances such as these have dictated retrenchment in an emergency.

Instability

Even in the United States, the strongest economy in the world, this state of affairs cannot last indefinitely. Two British economists, Professor M.J. Artis (University of Manchester) and Mr M.V. Posner (until last year the chairman of the Social Science Research Council) have given elegant explanations of the reasons in the autumn issue of *The Midland Bank Review*. Writing as members of a group of independent economists called the Clare group (after the Cambridge college of that name), they argue that the crucial question for lenders to the United States is not now whether they have confidence in the management of the US economy but whether they consider that other lenders will retain that sense. And since, on arithmetical grounds, the accumulation of both deficits cannot continue indefinitely, lenders have no choice but to second-guess other lenders. Sooner or later, the argument continues, the lenders will begin to lose their nerve, and then there will be only two choices for the US authorities — to increase interest rates still further in the possibly vain hope of continuing to finance the federal deficit or to let inflation run riot again. Either way, the economic recovery of the past two years would be stopped in its tracks.

This argument is not, of course, unfamiliar. It has been repeated endlessly in the past three years, especially in relation to the debts of the developing countries whose financial problems, serious enough a decade ago in the wake of the oil crises of 1983 and 1979, are aggravated as the rate of interest of foreign loans which cannot be serviced is converted to that prevailing in New York. So much, indeed, has been made of the instability of the present situation, and so little has come of these predictions of disaster, that it is tempting to think the general air of gloom must be mistaken. But that cannot be the case. Simple arithmetic decrees otherwise. The question is not whether both deficits can continue indefinitely, but when one or other or both of them must be eliminated, and how.

Obviously the best solution is that the US Administration should recognize the truth of what economists elsewhere are saying, that this state of affairs cannot persist indefinitely. The alternative to action is to wait for it to be resolved catastrophically. The Reagan Administration, whatever its faults, is fiscally well-managed and thus equipped to know when the time has come to change tack. Indeed, *The Midland Bank Review* (not the Clare group as such) takes the line that the change must come within the next twelve months. On the assumption that the administration (whatever its colour) may then have changed course, it guesses that interest rates in New York (and thus elsewhere) will have fallen by two per cent by the end of 1985 — and that the value of the dollar relative to other currencies will also have fallen by 20 per cent. Mr Walter Mondale, the Democratic candidate, has been promising such a change of tack, but for the time being the incumbent President dissents. What the economists are saying is that neither has a free choice. □

Space shuttle

Pentagon gains ground in battle for alternative

Washington

DESPITE a near-perfect maiden flight by the space shuttle orbiter *Discovery* last week, doubts over the shuttle's commercial prospects seem to be increasing. The shuttle successfully placed all its satellites into orbit, and the crew again demonstrated the possibilities of in-flight maintenance. But the day before *Discovery* touched down in California, an expert panel of the US National Research Council told Congress it was "not clear" that the shuttle would ever be competitive with an expendable launcher for military payloads and agreed with the air force's argument that a new launch vehicle would provide it with greater operational flexibility.

This leaves the National Aeronautical and Space Administration (NASA) with a major problem, because NASA had been counting on the military to pay for more than one third of projected future shuttle flights. If the US Air Force insists on developing its own expendable launcher for some of these flights, NASA may well have to increase its prices to civilian customers.

The National Research Council, the research arm of the National Academy of Sciences, was asked by appropriations committees of both houses of Congress to compare options for expendable launch vehicles capable of lifting large payloads into orbit. Its report echoes previous criticisms of the shuttle — main engine and fuel pump performance are still below specification, turn-around times are still too long and, says the committee, "a sustained engineering programme will be required to realize the full benefit" of the shuttle concept.

The air force has been unhappy with the shuttle for some time. Apart from the question of reliability, the Department of Defense has been concerned about the limited number of launch sites and the security difficulties should it wish to launch a sensitive satellite "without the public exposure that has become the norm in NASA flight operations". The research council panel also points out that in time of crisis the shuttle would have to overfly Soviet territory in order to put a satellite into a polar orbit suitable for reconnaissance: here, an unmanned launcher offers obvious advantages. And commercial companies might be happier if the Department of Defense were less likely to requisition planned shuttle flights, as it has the right to do.

The panel examined three possible expendable launchers being considered by the Department of Defense — the Titan 34D7/Centaur, the Atlas II and the SRB-

X, which is derived from the shuttle. All are based on existing technology. Any of these would be capable of meeting immediate requirements but none offers the possibility of expansion to carry loads much heavier than 10,000 pounds to geosynchronous orbit, the panel concludes. The cost of developing one of the alternatives would be around \$2,000 million.

The research council panel avoids addressing in detail how the prospect of a new competitor to the shuttle would affect NASA finances. NASA at present charges its customers only "out-of-pocket

expenses", although there is pressure for it to move to a full-cost policy. But already the European *Ariane* launcher "appears to be competitive" with the shuttle.

While apparently accepting all the military's arguments for a new expendable launcher, the National Research Council panel stops short of giving the project an unconditional endorsement. The main need, it concludes, is for further study of a new class of heavyweight launch vehicle capable of taking payloads of up to 450,000 pounds into low Earth orbit. Both NASA and the air force are already working on plans for such a system, thought likely to be necessary for President Reagan's "strategic defence initiative", better known as Star Wars. Such a system could be designed using motors developed for the shuttle programme with the aim of reducing costs without loss of reliability.

Tim Beardsley

British aerospace

Per atmosphaera ad astra

A BRITISH space shuttle? Surely a joke? Perhaps, perhaps not. A study by British Aerospace of a new concept for the launch of large payloads (up to 7 tonnes) into low Earth orbit comes as the British Government finds itself under increasing pressure from its high-technology companies and its European Space Agency partners to make a positive commitment to space or pass up for ever the chance to keep a presence on the "high frontier".

The new concept, dubbed HOTOL (Horizontal Take-Off and Land Satellite Launcher), is of a vehicle of aircraft type with the ability to take off horizontally and land on a conventional runway. The vehicle would use a combination of air breathing and rocket propulsion.

An ideal re-usable launch vehicle, says British Aerospace, should have no expendable parts (unlike the US space shuttle), should be capable of repeated operations with a minimum turn-around between missions, accommodate payloads in the 4-7 tonnes range and, if necessary, should be capable of unmanned operation.

At present vertical take-off into Earth orbit is impossible because even the highest-performance chemical fuels give a single-stage rocket insufficient impulse. A two-stage lift is essential, so that expensive equipment is irrevocably lost in re-entry or at sea.

British Aerospace proposes to overcome this apparently insurmountable difficulty by economizing on the supply of liquid oxygen, which may account for 85 per cent of the propellant mass of a booster rocket. If air scooped from the atmosphere were to supplement a rocket's supplies of liquid oxygen during the early phases of flight, its dead weight might be substantially reduced. Add wings for additional lift and there it is — HOTOL. British Aerospace believes the system capable of revolution-

alizing the space industry.

It is unlikely that Britain would have the resources to undertake such a major project on its own. If life were to be breathed into the idea, a collaborative effort, perhaps with other European countries, would seem the most prudent course to take.

It is just such a collaboration that European ministers are now pressing Britain to join, chiefly in the form of a European consortium to take part in the US manned space platform. Britain would



be expected to contribute an extra £15 million a year compared with its present spending of just £80 million a year (and France's £400 million), principally for membership of the European Space Agency.

British Aerospace is eager for government support for its space effort. But in the realm of fact rather than fancy, the company has announced a contract, from the US corporation Scott Science and Technology Inc., to design a Satellite Transfer Vehicle needed to carry satellites from shuttle orbits to geosynchronous orbit. The vehicle will be propelled by liquid fuels which, as well as being more efficient than solid fuel, will allow a range of burn strategies tailored to individual missions.

Marcus Chown

Acid rain

CEGB takes a pasting from MPs

BRITAIN should cut back drastically in its emissions of sulphur dioxide and other gases contributing to the problem of "acid rain", with most of the burden falling on the Central Electricity Generating Board (CEGB), according to a report published last week by an all-party committee of Members of Parliament. If the MPs recommendations were put into effect, they would force the CEGB to cut sulphur dioxide emissions by 85 per cent. This would cost "many thousands of millions of pounds" and raise electricity bills "up to 10 per cent", according to CEGB. Moreover, there is no guarantee that the measures (flue gas desulphurization, or in the long term fluidized bed combustion) to reduce sulphur emissions would have any commensurate effect on acid rain itself, the board claims.

But the MPs, after many months' taking of evidence from scientists and politicians in Britain, West Germany and Scandinavia, are convinced that scientific understanding is sufficiently advanced to demand action now. By this they mean that while a direct and proportional link between sulphur emissions and environmental damage is not scientifically proven, the case against sulphur dioxide is telling enough and the damage severe enough that action must be taken before all the evidence is in. In this they agree with a recently-published report by a committee of the House of Lords (see *Nature* 310, 535; 1984), but the bottom line of the Commons report — its demands — is far more punishing. And the MPs lay less stress than the Lords on the question of "linearity" between sulphur emissions and acid rain effects, a key scientific issue which is generally assumed to remain open. Moreover they quote modelling by Swedish professor of meteorology Henning Rodhe which supports linearity. Rodhe's models indicate that a decline in the acidity of Swedish rain since 1975 was attributable to a corresponding decline in European emissions — if depositions were linearly related to emissions.

Impressed by this and other evidence, and "appalled" by lack of action in Britain, the MPs say Britain must (among other measures):

- Join the "30 per cent club" of nations committed to reductions of 30 per cent in total national emissions of sulphur dioxide by 1990, a target to be achieved solely by cutbacks in CEGB emissions.
- Refit CEGB power stations to achieve a national 60 per cent reduction in sulphur dioxide emissions by 1995.

The second measure is broadly in line with an existing draft directive on "large combustion plant" from the Commission of the European Communities (CEC), but whereas CEC says that the measure should be applied to all plant of output greater

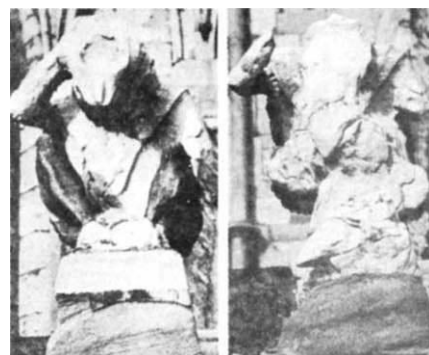
than 50 MW, the British MPs have singled out electricity supply for attack. "We don't want to affect manufacturing industry", a member of the Commons committee explained last week. As a result, he admitted, the measure would imply that CEGB cut its own emissions by 85 per cent, to achieve the net national reduction of 60 per cent.

Why should manufacturing industry be feather-bedded? To protect its fledgling rebirth in Britain, the MPs imply, and because CEGB's sulphur dioxide emissions have remained constant since 1970, whereas net British emissions have fallen 37 per cent. This fall was largely due to industry turning away from coal to natural gas (and to the recession).

Overall, it is difficult to avoid the impression that the MPs are singling out CEGB for punishment. Yet the punishment may be deserved. The MPs claim that CEGB "dismissed" the effects of acid emissions on stonework with the remark "to dissolve an inch of limestone would take 5,000–20,000 years", whereas, say the MPs, it is "well-understood" that dry deposition and the formation of calcium sulphate leads to the much more rapid process of flaking. In this context the report quotes the cathedral architect at Cologne as saying that none of the original stonework of Cologne cathedral will remain within five years, and that the constantly-repaired cathedral will become "a copy". The same process is at work in Britain, say the MPs.

In other areas CEGB has "begun work only two years ago" or "made no attempt to monitor effects". And, say the MPs, while CEGB is spending £1.5 million a year on research on the environmental effects of acid rain, it is spending only £200,000 on research on flue gas desulphurization "largely on paper studies of foreign systems".

As for scientific evidence, the committee claims it took pains to get balanced and detailed views in the UK and abroad. "I shouldn't comment on the Lords", said committee chairman Sir Hugh Rossi, "but they didn't do their homework — they



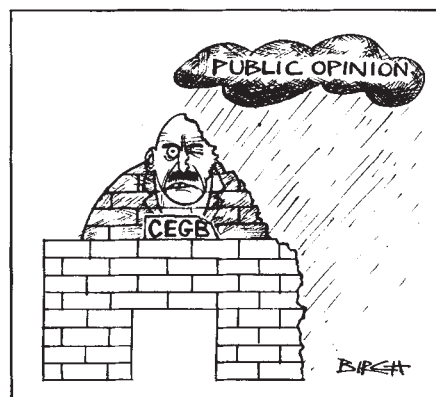
Stonework on Lincoln Cathedral, in the plume path of Trentside power stations. "Despite... the knowledge of CEGB research staff that the cathedral was suffering from the effects of sulphur dioxide, CEGB has made no effort to monitor those effects", say British MPs in a new report on acid rain. On the left, a photograph from 1910; on the right, 1984.

didn't go abroad". The Lords' report was consequently superficial, he implied.

The evidence of UK Natural Environment Research Council (NERC) scientists impressed the MPs. These scientists "independent of any manufacturing or industrial interests... told us that scientific work had advanced far enough for a decision to be taken". The minutes of evidence show, indeed, that NERC secretary John Bowman volunteered that although the next five years' research would provide "a good many answers to the questions you have been putting to us... I do not think that should be used as an argument for doing nothing now".

And as for the liming of acidified lakes in Scandinavia — as recommended by the Watt committee on energy (see *Nature* 310, 535; 1984) — this showed ignorance of Scandinavian research, and mere geography, which made liming impractical even as a short-term measure, committee members said last week. "Anyone who has flown over southern Norway and seen the number of damaged lakes in remote areas where there is no indigenous source of lime would realize this" the report says.

The only solution is for Britain to put its own house in order, say the MPs. But will their advice be taken? The next political step in Britain will be a debate on the report. According to Sir Hugh Rossi the government has promised such a debate before the end of the year. **Robert Walgate** • Britain's Social Democratic Party (SDP) announced their own solution to the problem last week: energy conservation. "Substantial reductions [in sulphur dioxide emissions] could be achieved rapidly even without the costly installation of desulphurization equipment in power stations if the government invested more in energy efficiency measures. A five per cent cut in electricity generation would enable Britain to meet the Select Committee's targets by the early 1990s, at half the cost estimated by the CEGB. It would also improve the competitiveness of industry", the SDP claims. □



Laboratory animals

Animal rights lobby cashes in

THERE is still no word from the British Government on the progress of its new animal experimentation legislation. More than a year has passed since the release of the White Paper — "Scientific Procedures on Living Animals" (see *Nature* 303, 191; 1984) — containing draft proposals for the replacement of the Cruelty to Animals Act of 1876 with more effective and less anachronistic controls. In that year, the views and critical comments of concerned organizations have been collected and assimilated. Moderate animal rights groups are now hoping that a suitable response, in the form of a supplementary White Paper, will be published by the end of the year.

Meanwhile, the more extreme animal rights groups, including the Animal Liberation Front (ALF), have stepped up their campaign against animal experimentation with paramilitary style attacks on selected institutions. Government laboratories have been forcibly entered, equipment damaged and records essential to research stolen. Pharmaceutical companies have also suffered harassment and intimidation of their employees. And Mr David Mellor, Under-Secretary at the Home Office and the man principally responsible for preparing the new legislation, has become the object of escalating harassment which, he says, has included threats on his life. Meanwhile, a coalition of organizations including Animal Aid and the British Union for the Abolition of Vivisection, has drawn up a list of minimum requirements which, it says, must be part of the legislation to replace the archaic 1876 act. It wants to see a complete ban on tests of cosmetics, alcohol and tobacco, behavioural and psychological tests, warfare experiments and, more specifically, two routine tests it considers particularly offensive: the Draize eye test in which irritants are put in the eyes, usually of rabbits, until damage is caused, and the so-called LD50 toxicity test in which animals are given increasing doses of a test substance until half have died.

The coalition is unlikely to be satisfied with the proposed measures which, in order not to hamstring British companies by prohibiting experiments that competing foreign companies would be free to carry out, avoids a complete research ban. The proposals differ from the old act in the following ways:

- The 1876 law, which only governs experiments, will be extended in scope to address procedures such as the production of antisera and the passaging of tumours.
- A statutory Animal Procedures Committee will replace the previous non-statutory body.
- Laboratory animals will have to be obtained from registered establishments, outlawing the use of strays.

- The 1876 law required all animals recovering from anaesthesia to be compulsorily killed but, today, some surgical procedures are relatively mild and anaesthesia is used principally to immobilize a subject. This requirement is to be waived in such circumstances.

- The conditions in which animals are maintained will be regulated.

- Home Office inspectors will need to be satisfied that a procedure is justifiable and that no satisfactory alternative to the use of animals is feasible, that the minimum number of animals is used and that the least possible suffering is caused.

The Royal Society for the Prevention of Cruelty to Animals (RSPCA) is generally critical of these proposals. The White Paper advocates the continuation of the present rule that "if an animal is found to be suffering severe pain which is likely to endure, it shall at once be killed". But, RSPCA points out, since the Home Office itself admits it cannot define the terms "pain", "severe" or "enduring", the inclusion of these words in such a pivotal role undermines the credibility of the proposals. Other organizations are also insistent that the pain question be dealt with. The Fund for the Replacement of Animals in Medical Experiments (FRAME) is committed to reducing animal experiments to a minimum and to finding alternatives to animals. In a study, prepared in conjunction with the British Veterinary Association (BVA) and the Committee for the Reform of Animal Experimentation (CRAE) and supplied to the Home Office, it distinguishes two types of pain: unexpected pain inflicted during an

Britain leads the way

DESPITE the flak aimed at the government by animal rights organizations, Britain is ahead of most other European countries in the area of animal experimentation reform. The 21-nation Council of Europe has been trying, since 1978, to perfect a "Convention for the protection of animals used for experimental purposes" (*Nature* 302, 284; 1983) and the convention is likely to be less strict than the British law. Nevertheless, a European accord on the political horizon could go some way to allaying the fears of pharmaceutical companies that what they perceive as an over-zealous British initiative.

The ageing British act also has admirers in the United States. In a Commentary appearing in *Nature* later this month, an animal rights campaigner argues that the British approach to animal experimentation is much more humane than the US approach. Two bills aiming to improve matters are at present bogged down in the US Congress, with the powerful industrial lobby content to keep them there. □

experiment, and pain and suffering which is the inevitable consequence of an experiment. In the former situation, FRAME says, the animal should be killed as required by the 1876 act. In the latter situation, permission should be given only in very special circumstances when the experiment is of great importance to human or animal health.

Both the RSPCA and FRAME are unwilling to be too critical of the Home Office until they see its revised proposals and can assess to what extent their advice has been heeded. The new bill could reach Parliament during next year or in 1986.

Marcus Chown

Bird books safe

Washington

A UNIQUE collection of environmental and natural history documents, including the only known intact copy of the "elephant folio" edition of John Audubon's *Birds of America*, has been granted a reprieve from the auction block. The National Audubon Society had planned to close the library and sell off part of it to raise funds, but the society has now changed its mind after word of the plan leaked out and provoked a minor squall among members and the American Library Association. Many were upset that the board of directors of the society did not consult its membership before taking the decision at its May meeting, and that the society has in fact still made no official announcement on the subject.

According to the library association, the collection, which is housed in the society's New York offices, contains many works on ornithology, wildlife preservation and conservation which can be found together in

no other library. It also includes a number of unduplicated nineteenth century works.

The library dispute appears to be part of larger tensions that have been growing between the society's membership and its staff on the one hand and its leadership on the other. Under the presidency of Russell Peterson, the society has become increasingly involved in what is known in the trade association business as "government affairs", that is, lobbying. Peterson attempted to move the entire office to Washington several years ago, but abandoned the plan. Peterson has also involved the society in such issues as global population and nuclear war. Many staff members are known to be displeased with what they see as a swelling bureaucracy — including some 25 vice-presidents — which has grown at the expense of basic programmes.

Although the library will remain intact for the time being, it has been closed to the public and its full-time librarian assigned to new duties within the society.

Stephen Budiansky

Oceanography

Excitement on the seabed

THE US oceanographic and geological community has been "set alight" by a quarter-million-square-mile survey of the Pacific Ocean floor off the west coast of the United States — a survey, completed three weeks ago with the British side-scanning sonar system GLORIA, of the 200-mile exclusive economic zone (EEZ) declared by President Reagan last year under the international Law of the Sea. The survey, says Dr Jim Gardner of the US Geological Survey (USGS) at Menlo Park, has for the first time provided a "road map" of the whole area stretching from the Mexican border in the south to the Canadian in the north, and outwards into the Pacific from the edge of the narrow continental shelf. This map reveals some 200 previously unknown volcanic sea-mounts, "spectacular canyons" 300–500 km long — apparently carrying meandering rivers of sediment along the ocean floor — and almost every kind of plate tectonic boundary.

Economically, the sea-mounts may offer sources of strategic minerals, and the canyons and ocean-floor fans will interest the oil industry both as models of older sources of oil, and as possible drilling sites. There are no indications of sea-floor manganese nodules, but these were not expected so far north.

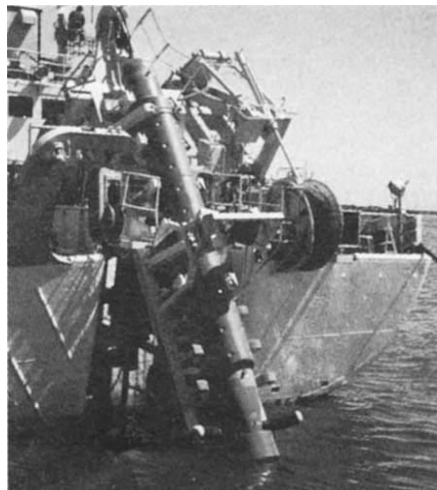
The survey, says Gardner, who headed the American side of the US-British team that did the work, will lead to half-a-dozen priority research projects for more detailed investigation, and already \$60 million is inscribed in the USGS budget for fiscal year 1986 (yet to be approved) to pay for the work (over five years). This will be done jointly with universities, Gardner says.

In Britain, the excitement matches Gardner's, with the extra twist that the mission's success offers a real opportunity for British enterprise. GLORIA was developed at the Institute of Oceanographic Sciences (IOS) at Wormley, Surrey, and has a uniquely long range, which makes it extremely cost-effective for the survey of the 200-mile EEZs which almost every nation has declared since the 'Law of the Sea' agreement. According to Neil Kenyon of IOS, who with another British oceanographer Douglas Masson took charge of the British side of the recent GLORIA mission, GLORIA can perform an area survey 10 times faster than any competing system. It can survey an area half the size of Wales every day at a cost of \$5 per square mile, IOS claims.

As a result there is now likely to be a flood of interest in using the torpedo-like instrument to survey EEZs around the world. Already USGS is negotiating for a further five years' work with GLORIA, says Gardner, starting next year in the Gulf of Mexico, and later covering Alaska and Hawaii; and, says Dr Tony Laughton, the

director of IOS, to judge by reaction from the recent second international hydrographic technical conference in Plymouth, there are already indications of real interest in many other countries.

But that leaves IOS with an interesting problem. There is only one GLORIA. The USGS programme, if agreed, would occupy it nearly full-time. Apart from the interest of other nations, IOS scientists must have their own time with the instrument. And IOS funds, provided through



GLORIA — geological long-range inclined ASDIC — is an acronym of an acronym. Dating from the second world war, ASDIC stood for "anti-submarine detection investigation committee". The acronym came to be used to describe the acoustic echo system developed in Britain to detect German U-boats. The achievements of GLORIA represent a 40-year development of ASDIC. The photograph shows GLORIA being launched from the Farnella research vessel — a converted trawler.

the Natural Environment Research Council (NERC), are so short it would seem impossible to find the financial and human resources to build a second instrument, costing around £1 million. "If we were a commercial company, we would borrow money to build a new GLORIA on the prospect of making a profit", said Lawton. And, says IOS administrator Dr Tony Rees, "with ten GLORIAS we could cover the world in ten years".

Treasury rules, under which NERC (and hence IOS) operate, make borrowing difficult. But since the present government is in favour of enterprise, Dr Lawton is hopeful something might be arranged. Other possibilities he is considering are to establish a private company to exploit GLORIA or to license the technology to an existing company. However, too much has been published about the instrument for it to be patentable, he thinks. The potential profits are large. But IOS will have to strike the right balance between commerce and science, says Lawton. "It clearly poses some big problems in terms of our role."

Robert Walgate

European solar energy

Off to sunny Sicily

Brussels

HELPED by the European Commission, a consortium of European companies will next month launch an experiment, in Sicily, to test four types of photovoltaic cells "in the field" for the first time. According to Commission officials, the data collected could play an important role in developing cheaper and more efficient solar cells.

The project will compare fixed and sun-tracking systems of monocrystalline and polycrystalline silicon modules, and two alternative modules: flat-plate amorphous silicon cells and concentrator gallium arsenide cells. The electricity produced by the 5 kW systems will be used in small local applications. The companies involved in the design and manufacture of the components are the Italian companies

BA gets under way

THE 146th annual meeting of the British Association for the Advancement of Science (BA) opened at the University of East Anglia in Norwich on Monday, with over 3,000 visitors expected — 700 more than last year's conference in Brighton. In recent years the BA has been seeking a new identity; it may have found it at Norwich in a mix of science popularization, science politics (whither Britain?), due concern for the place of science in the developing world, and increasing efforts to link science and industry in the minds of its visitors.



To paraphrase the outgoing president Sir Alastair Pilkington (the glassmaker) the BA represents an effort to put science to work in Britain: it has a utilitarian, constructive goal. According to the incoming president, biochemist Sir Hans Kornberg — a man closer to the cuts that British science has suffered recently — next year's BA meeting at the University of Strathclyde will see a greater emphasis on basic science (and perhaps a little more polemic on its behalf) than at Norwich.

But BA officers are very pleased with the £450,000 that Norwich has raised locally to support the meeting (covering more than half its costs) and with the buoyancy of the BA young scientists scheme (BAYS), with its 130 local branches (ever increasing) which hold local meetings throughout the year. And this year there is a new departure: the BA is launching a glossy new magazine, *Link-up*, edited by John Stansell, which will aim to link university science with industrial problems.

Robert Walgate

ENEL and Pragma, and MAN and Siemens of West Germany. Only a dozen or so European companies have been seriously developing solar energy components, including photovoltaic cells. In 1981, Europe had only a 12 per cent share of the world photovoltaics markets which then totalled 6 MWP (megawatts at peak). According to Commission estimates, the world market is expected to reach 400 MWP by 1990, and Commission officials believe that Europe could corner up to 40 per cent of that market. But Europe's main competitors, the United States and Japan, have a head start because of the extensive governmental support given to work on solar energy in those countries.

The solar energy industry is well-established in the United States. The photovoltaics market is worth an annual turnover of \$55 million to the fifteen companies involved in component and systems production. The US research and development programme is extensive, while Japanese companies are forging ahead with the development of large-area low-cost amorphous silicon cells, encouraged by a MITI subsidiary, the New Energy Development Organization (NEDO).

National non-nuclear energy budgets in Europe are less generous to research in this field. Tax incentives to encourage the production of electricity using solar energy are becoming more and more frequent in the United States, but in Europe such stimulants have so far only been introduced in Denmark.

The European Community's non-nuclear energy research and development programme has contributed to bringing the cost of silicon cells down to one-fifth of their 1973 cost. According to those working in the field, the cost could be down to one dollar per watt by the year 2000.

However, the community's second pluri-annual programme came to an end in June 1983, while the ambitious budget of 379 million European Currency Units (2 ECU = £1) which the European Commission proposed for the third programme — a tripling of the previous budget — has been held up by the Community's unresolved budgetary wrangles.

In the meantime the delay continues to take its toll of research operations. Far-seeing officials earmarked funds from the 1979-83 budget to keep certain projects ticking over. But lack of funds in other projects will mean that research teams may be moved to other fields. Calls for tenders have also been delayed, so that it may be another year before work on new contracts can get under way.

In addition, extra investments allocated to the Community's advanced information technology programme (Esprit), launched this year, mean that cuts have to be made in other areas in 1984 and 1985. Solar energy research is no exception. The three largest EEC member states, West

Germany, France and the United Kingdom, have opposed the draft budget proposed by the Commission at successive research councils. The proposal is not likely to fare any better when the ten's research ministers take their next look at it in mid-October.

The potential of photovoltaics is reflected in the funds earmarked for it under the third non-nuclear energy research and development programme proposed by the Commission — half of the solar energy sub-programme (80 million ECU) which also includes developing solar architecture, passive solar heating and cooling systems, and instruments for analysis, testing solar energy systems and looking at the problems of storage and performance.

As far as photovoltaics are concerned, the Commission wants to lower the costs of cells and develop competitive large-area power supply systems during the next four years. The second area of priority is biomass. It is estimated that the conversion of biomass could eventually provide 5 per cent of the Community's energy requirement, and half a million extra jobs. The Community has a 38 per cent energy deficit at the moment. Eight of the ten member countries have to import between 48 per cent (Greece) and 81 per cent (Italy) of their energy requirement from abroad — the two energy exporters are the United Kingdom and the Netherlands.

The third area on which research is to be concentrated is geothermal energy. The Commission has proposed spending 39 million ECU to develop low temperature source applications, and look at the problems of reservoir management, fluid extraction, energy conversion and develop the feasibility of the hot dry rocks process.

The final area of research and development is wind energy. In addition to monitoring the performance of some 200 wind generators, which are scattered throughout the ten member states, Community research would be centred on the development of multiwatt machines, the improvement of blade design and engineering, improving the technology of small and medium sized machines, solving the problems of site aerodynamics, sub-system concepts and integration into the overall electrical supply system.

Anna Lubinska

Science in India

In the special issue on science in India (*Nature* 308, 581-600; 1984), in an article dealing with the administration of research (pp.591 *et seq.*), there is a reference to an academic's complaint that he had to negotiate with his university to spend Rs100. The same paragraph mentioned the Madurai Kamaraj University, and the vice-chancellor, Dr J. Ramachandran, asks us to point out that the reference was not to his university. □

Mongolia

New man backs science

THE new general secretary of the Central Committee of the Mongolian People's Revolutionary Party, Dr Jambyn Batmonh, is something of a rarity among communist leaders. The usual route to political power involves rising through the ranks of the party or military apparatus. But Dr Jambyn is a former academic with good links with the scientific community.

Educated at the Mongolian State University and at the Academy of Social Sciences of the Central Committee of the Communist Party of the Soviet Union, Dr Batmonh worked for more than twenty years in Mongolia's higher education system. In 1967 he became pro-rector, and subsequently rector, of the Mongolian State University.

In 1973, he transferred to party work as head of the central committee's Department of Science and Education. In 1974, he became chairman of the Council of Mini-



Dr Batmonh — academic makes good.

sters of the Mongolian People's Republic. Given his background, it is not surprising that his inaugural speech as general secretary stressed the importance of science and technology in raising the efficiency of Mongolian agriculture and the need to improve education and health facilities.

The son of north-Mongolian cattleherders, Dr Batmonh's *curriculum vitae* illustrates the rapid development of education in Mongolia which (apart from lamasery schools) began only five years before his birth with the foundation of a 40-pupil elementary school in Urga (now Ulan Bator). The Mongolian State University was founded only in 1942 and the Mongolian Academy of Sciences in 1941. As a result of the lack of opportunity at home, a strong tradition has developed that Mongolian scholars do at least a part of their postgraduate studies in the Soviet Union, and in many fields of research, including the application of radioisotopes, the development of the Mongolian chemical industry and the geophysical survey, Soviet scientists continue to play an important role.

Vera Rich

Siberia

Canals to feed saline lake

SOVIET plans to divert the north-flowing Siberian rivers southward have received a new impetus, with the launch of a scheme to replenish the waters of the landlocked Aral Sea. Increasing irrigation demands on the Rivers Amudarya and Syrdarya which feed the Aral have led to a drop in the water level of 3 metres in the past two decades. The consequent rise in salinity has had an adverse effect on the fish-stocks of the lake.

As the Aral basin is the main source of Soviet cotton and the 1982 Food Programme envisages further irrigation projects in the area, a massive rescue operation has been planned, requiring the construction of a 2,550 km canal from the confluence of the Ob' and Irtysh rivers to the Amudarya. This will not only replenish the Aral Sea to its optimum level, but also permit the irrigation of an additional 4 million hectares of land. The fall in water-level is undoubtedly worrying to ecologists, who, until the canal is ready, plan to regulate evaporation losses by building two dams, which will cut off Adzhibai Bay and the gulf known as the Little Sea and thus keep those areas close to the river mouths at a salinity level suitable for fisheries.

Dam-construction in Soviet seas is, however, a sensitive issue. A dam completed five years ago, which was meant to halt evaporation losses in the Caspian Sea has instead caused disastrous changes in the subterranean brines which form the feedstock of the Karbogazsulfat chemical industry, and have turned the region east of the gulf into a salt desert. (The Caspian level, meanwhile, has risen through apparently natural causes.)

The proposed use of the Irtysh water for the diversion scheme, too, is not without its problems. Although the proposed canal will draw off only 6 per cent of the Ob'-Irtysh water resources, even this small proportion could raise difficulties. Recently there has been a continuing dispute on the upper Irtysh between the farmers, fishermen and river-boatmen who depend on a plentiful water-supply, and the Kazakhstan Ministry of Land Reclamation and Water Resources which withheld supplies at the request of the electrical generating authorities. According to *Kazakhstanskaya Pravda*, there have been serious planning errors in the construction of hydroelectric works on the upper Irtysh which will require further major engineering works to put right. Yet all public statements on the proposed canal to the Amudarya tacitly assume that the Ob' and Irtysh have no difficulties with their "water budgets".

The emphasis on the small percentage of water to be drawn off by the canal, and the consequent assurances that its diversion will not affect the ecology of the region is a fairly new approach. Earlier plans spoke

grandiosely of turning whole rivers back in their courses. When, however, the schemes were revived in the early 1970s, there was a worldwide outcry from ecologists, who feared that the loss of water to the Arctic Ocean might change the world's weather patterns. The Soviet planners issued vehement denials that such a disaster might occur, but postponed their plans until further surveys could be carried out.

As a result, they have introduced a major modification into the "diversions"

announced during the 1970s. The plan originally involved replenishing the feeder rivers of both the Azov and Caspian Seas. To do this, however, would necessitate the transfer of more than 20 km³ of water a year, too great an amount, according to planners, for the environmental changes to be predictable. (The Amudarya canal will transfer 27.2 km³ a year.) Accordingly, it was decided to concentrate on the Caspian, not only because its present "water-budget" is still fairly close to equilibrium as regards inflow but also because of the particular economic importance of the Caspian as the largest producer of sturgeon in the world.

Vera Rich

UK research

Councils hunting for heads

BRITISH research councils appear to be increasingly hamstrung in their attempts to recruit new chief executives, at least in part because suitable candidates now consider senior positions with the research councils to be uncomfortable beds of nails. Three of the five councils will need to appoint new chairmen within the next twelve months.

The Natural Environment Research Council is in the most urgent need now that its chairman for the past three years, Sir Hermann Bondi, has become Master of Churchill College, Cambridge. Apparently the council has been rebuffed by the two candidates first approached, but has now recommended (to the Secretary of State for Education and Science) that Dr Hugh Fish, chairman of the London Water Board and

one of its own members, should be appointed chairman on a short-term basis.

The Agricultural and Food Research Council, whose secretary Sir Ralph Riley is on the point of retirement, is by contrast using the services of an executive recruitment agency to appoint a successor. By all accounts, however, the Science and Engineering Research Council plans to go one substantial step further and to use a head-hunting company as a means of identifying possible candidates. In this case, there is a suitable breathing space. The present chairman of the council, Professor John Kingman, is due to become vice-chancellor of the University of Bristol only at the beginning of the academic year 1985-86. □

Defence department money on offer

Washington,

THE US Department of Defense is putting its money where its mouth has been on biological warfare, and a major beneficiary may be academic researchers. A series of full-page advertisements have recently appeared in *Science* magazine, placed by the US Army Medical Research Acquisition Agency, calling for proposals in a broad range of areas related to protection against biological agents.

A spokesman for the US Army Medical Research Institute of Infectious Diseases (USAMRIID), the old biological warfare establishment at Fort Detrick, Maryland, said that the solicitations are part of a general effort to expand its research programmes on biological defence in four areas: parasitic diseases, low-molecular-weight toxins, rickettsial diseases and bacterial diseases. The most recent advertisement seeks research proposals on biochemical, immunological, genetic, pathophysiological and therapeutic studies of bacterial diseases, with special emphasis on anthrax, botulin, diarrhoeal diseases and "other bacteria or toxins capable of eroding combat strengths" or "agents of potential biological warfare importance".

The USAMRIID spokesman said that the advertising campaign was simply a way of letting scientists who are probably already familiar with the institute's work learn how they can submit proposals. All of the work is unclassified.

Support for USAMRIID has risen dramatically in the past few years, from \$17 million in fiscal year 1982 to approximately \$40 million this year. The current plan is to double that figure over the next three years. The entire Army Medical Research Command, of which USAMRIID is a part, is now spending more than \$100 million a year on extramural research, although this figure includes not only biological warfare defence research but other topics relating to soldiers' health.

The USAMRIID spokesman attributed the new emphasis on medical research both to a heightened concern with preparedness — the Secretary of Defense, Caspar Weinberger, has cited medical care as a critical element in US war-fighting capability — and also to the biological warfare threat perceived in the Soviet Union's supposed proxy use of "yellow rain" in South-East Asia.

Stephen Budiansky

US agricultural research

Competition reaps rewards

Washington

A STUDY of US agricultural research now being circulated to colleges and universities offers encouragement to scientists and may pave the way for more coherent planning. The report* emphasizes the need for improved communications between Congress and the executive so that scientific projects do not become hostages to political fortune, and may lead to an expansion of competitive grant funding. This would bring benefits to the underdeveloped plant biotechnology research programme.

The timing of the report is apt: next year's agriculture appropriations bill remains jammed in Congress. The Senate has restored the administration's \$50 million budget request for the Department of Agriculture's competitive grants programme after the House sought to cut this to \$17.5 million. The \$50 million includes \$28.5 million allocated to the administration's new biotechnology initiative. Although no conference committee has yet been appointed to bridge the gap, much behind-the-scenes negotiating is going on. But despite the generally favourable light in which science is regarded in Congress, prominent figures in the House consider basic research in agriculture irrelevant to the immediate needs of farmers.

The report now being circulated was prepared under contract for the Department of Agriculture as an "action research programme", involving scores of interviews. It is heavy with sociological theorizing and even ventures some speculations on philosophical issues: for example, the "logical positivistic" underpinnings of agricultural research are blamed for the "paradox of faith in science leading to doubt and distrust". But there are also some more concrete suggestions, chief among which is a proposal for a new National Agricultural Issues Commission based within the National Academy of Sciences which would define long-term research goals.

Ironically, that proposal is the one that seems least likely to get anywhere. A majority of the key decision makers who reviewed a draft of the report felt that two existing bodies — the Joint Council on Food and Agricultural Sciences and the Users Advisory Board — could do the job.

Many of the report's other conclusions have been well received, although a proposal for new centres of excellence in agricultural research is contentious. The report urges efforts to broaden recruitment to bring in more students from non-farm backgrounds, and argues for increased prominence and status for scientists. A separate study being completed by Professor Irwin Feller of Pennsylvania State University, shortly to be delivered to the agriculture department, is thought to

have a similarly up-beat approach towards future contributions of research.

The combined impact of both studies is likely to have a "slow but significant effect" on policy, according to Dr John Jordan, administrator of the agriculture department's cooperative state research service. The activity of recent months has brought together congressmen and

scientists "who would never normally see each other". The use of competitive grants to fund research is now likely to increase, thus allowing major research centres outside the land-grant system to make bids. And even the land-grant colleges are starting to make more use of competitive schemes and joint schemes with non-land-grant institutions, says Jordan.

Tim Beardsley

**The Paradox of Success: The Impact of Priority Setting in Agricultural Research and Extension, (US Department of Agriculture).*

New US genetics centre goes west

San Francisco

A NEW research centre concerned with genetic engineering of crop plants has been established jointly by the US Department of Agriculture (USDA) and the University of California. The centre hopes to attract university and industry researchers from all over the world to work on basic research projects in plant genetics and gene expression.

The Plant Gene Expression Center, with an initial annual budget of \$4 million, will be housed in USDA's Western Regional Research Center in Albany, near the Berkeley campus of the university. The facility has laboratory space for about 40 researchers and is expected to be operational within a year. Ten senior scientists will form the foundation of the centre, including two senior scientist programme leaders holding university appointments. The remaining eight core scientists will hold positions with USDA's Agricultural Research Service (ARS).

The core group will be supported by a consortium of public and private researchers. The consortium serves as a

source of expertise and provides an important link with other research institutions and industry. In addition, a science advisory committee will be formed to help set research priorities.

The centre will investigate basic questions in plant molecular biology, using and further developing new tools of plant genetic engineering. The centre wants to make its research findings available to anyone, including those interested in developing marketable products. Details of publication policy have not yet been worked out, according to Gerald Still, acting director of the centre. Usually the centre will follow the ARS practice of establishing public patents and refusing industry support that stipulates secrecy and exclusive rights. Dr Still, formerly National Program Leader at ARS in Beltsville, Maryland, has the task of coordinating research activities, arranging cooperative agreements and recruiting financial support. He hopes to supplement the annual budget provided by ARS with grants from other public and private agencies and industry. Bruni Kobbe

Nature index of biotechnology stocks

12-Month high	12-Month low	Company	Close previous month	Close 31 August	Change
14	6	Biogen (Switzerland)	6¾	8	+ 1¼
2	1	Bio-Logicals (Canada)	1⅞	1¼	+ ⅞
14⅜	7⅞	Bio-Response (USA)	8⅞	9½	+ ⅞
14	9¾	Cetus (USA)	9⅞	11⅜	+ 2
10⅞	4¼	Collaborative Research (USA)	5¼	6¼	+ 1
19⅞	11½	Damon (USA)	13	14½	+ 1½
26¼	11¾	Enzo-Biochem (USA)	12½	14¼	+ 1¾
10⅞	4¼	Flow General (USA)	4¾	5⅞	+ ⅞
42¼	28¾	Genentech (USA)	33	33¼	+ ¼
10¾	4½	Genetic Systems (USA)	5	6⅞	+ 1⅞
17¼	8¼	Genex (USA)	11⅞	12	+ ⅞
23	11	Hybritech (USA)	11¼	14¼	+ 3
16¼	7¾	Molecular Genetics (USA)	8¾	10¼	+ 1½
15½	8¼	Monoclonal Antibodies (USA)	8¼	10	+ 1¾
60⅞	39	Novo Industri A/S (Denmark)	40⅞	37¼	- 3⅞
22¾	14½	Pharmacia (Sweden)	17⅞	19	+ 1⅞

Closing prices are for the last Friday of the month. For over-the-counter stocks, bid price is quoted; for stocks on the American and New York exchanges, the transaction price. *Nature's* weighted index of biotechnology stocks stood at 149 on 31 August, compared with 138 a month earlier. Data from E.F. Hutton, Inc.

Roman food not so fast

SIR — As a pioneer in the study of animal remains from archaeological sites, I counsel caution in concluding that a high proportion of pig bones indicates the decline of Rome into a fast-food empire (*Nature* 17 May, p.211). First, the bones indicate the proportions in which the different species were eaten rather than kept. Although in a peasant economy the two are probably the same, it is noteworthy that whereas the Cistercian monks kept thousands of sheep but relatively few cattle, 90 per cent of the medieval bone remains from Kirkstall Abbey were from cattle¹.

Although it is true that the greatest proportion of pig bones from a Roman site in Britain (for comparison) was 26 per cent, the figures of 40 per cent and even 65 per cent pigs quoted by Hodges from Italy hardly indicate overwhelming predominance when one notes that cattle remains ranged up to 78 per cent and sheep remains up to 95 per cent on Roman sites, again in Britain².

The term "fast food" used by Hodges is used in a modern context for food preparation rather than for food production. Since the pig was a scavenger in the past, pork was certainly cheap to produce, but not "fast" or "short term" as Hodges implies, since until after the Middle Ages pigs were slow-maturing animals. If the production of pigmeat is relatively "fast" today (as a major component of "fast" or convenience foods) it is because pigs have been bred for early maturity and are fed concentrated cereal-based diets.

M.L. RYDER

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1. Ryder, M.L. *Animal bones in Archaeology* 2nd edn (Blackwell, Oxford, 1969).
2. Ryder, M.L. "Livestock" in *The Agrarian History of England and Wales Vol. 1, Pt 1* (ed. Piggott, S.) (Cambridge University Press, 1981).

Richard Hodges replies:

SIR — Dr Ryder is making a mountain out of a molehill. My short review in *Nature* placed in a wider historical context a collection of recently published studies by archaeozoologists, in whom I have every confidence.

First, I made exactly the point (the main thrust of my note) that it was consumption of pig rather than any assumptions about production directly that made the data so historically interesting. I agree with him. However, I suspect his Kirkstall Abbey example is a poor illustration of his point, since I would imagine his high cattle bone is merely an indication of the sampling procedures (or lack of them) used when the site was excavated.

Second, I do not follow his point about Roman sites in Britain. To make such comparisons without considering historical and ecological contexts for the sites concerned

is quite unscientific.

Third, I like the term "fast-food", suggested to me by your assistant editor. Of course, Ryder is strictly right. The term should have been qualified — and would have been in a long article — by precisely the comments he makes, effectively endorsing the reason for drawing attention to high pig numbers in the first place.

RICHARD HODGES

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Cancer incidence at Sellafield

SIR — The investigation by Sir Douglas Black (see *Nature* 26 July, p.263) into the alleged increased incidence of cancer in West Cumbria usefully suggested what further research is needed, especially the measurement of radiation dose actually received and whether metabolic differences between children and adults alter the effects of radiation. However, on the

Ward rank order	Cases	Children	Poisson probability	Rate per 1,000
1*	4	411	0.0001	9.73
2	4	976	0.0032	4.09
3	2	144	0.0036	13.88
4	4	1,207	0.0068	3.31
5	4	1,353	0.0100	2.95

Top five wards ranked by Poisson probability for incidence of lymphoid malignancy in under 15 year olds, 1968–82 (from Craft *et al.* ²).

*Seascale.

evidence already available, doubts must be raised about the report's judgement that the incidence of leukaemia in Seascale is "unusual but not unique".

The report examines the occurrence of lymphoid malignancy, in children under 15, in the 765 electoral wards of Cumbria, from 1968 to 1982. Incidence in the wards was ranked by rate per 1,000. Seascale (9.73/1,000) is ranked third. Although well above average (0.61/1,000), Seascale appears to lie within the normal pattern of variation.

However, rate so measured is an uninformative index. As most wards have only 0 or 1 cases, all that is reflected in rate is ward size, which varies twenty-fold (from 100 to 2,000). A more appropriate measure is the cumulative Poisson probability. This gives the probability of occurrence in a ward of 0, 1, 2, . . . cases, if the cases are randomly distributed in the region. The incidence seen in Seascale is 1.3×10^{-4} , which when ranked lies first (see table and ref. 2). This incidence is expected to occur once in a sample of 8,000 wards.

Even more disturbing are the two further cases of lymphoma registered in Seascale in 1983 (ref. 1). By updating the survey to

1983, the Poisson probability for such an occurrence falls to approximately 1.0×10^{-6} . This can only be described as unique, lying well beyond the expected variation.

The two simple points raised here must make the "qualified reassurance" given by the Black report less acceptable. Seascale has an extraordinarily high incidence of lymphoid malignancy. Its proximity to the Sellafield reprocessing plant, a potential source of mutagenic radiation, is disquieting.

ANDREW POMIANKOWSKI

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1. Report of the Independent Advisory Group, *Investigation of the Possible Increased Incidence of Cancer in West Cumbria* (HMSO, London, 1984).

2. Craft, A. W. Openshaw, S. & Birch, J. *Lancet* ii, 96-97 (1984).

Too many papers?

SIR — For some years there has been talk of there being too many scientific journals but now it appears that there are too many papers. An informal sampling suggests that, in science and medicine, journals of reasonable status are experiencing an increase of around 10 per cent in submissions while editors in the area of whole organism biology or traditional life science disciplines have reported increases of up to 30 per cent, and even 50 per cent in the case of certain high status titles.

There are several possible explanations.

(1) A demographic factor; as a result of the growth in tertiary education in the 1960s, a large number of scientists are now in their most productive period and could be making a last effort to gain promotion. (2) Support of research in some areas, especially "soft" science, has been severely cut, heightening the struggle for grants. (3) Information technology has made it easier to store and process research data. This could be having an effect in astrophysics and geophysics in particular. (4) The decline in the publication of multi-author review volumes, resulting from library cut-backs, may have diverted longer review articles, or just the writing effort involved, towards refereed journals.

At present, smaller journals are being forced to restrict page budgets and there is the prospect of worthwhile if specialized papers going unpublished. Whatever the underlying reasons for the apparent increase in papers, the signs indicate a developing problem in the publication of research, especially in non-commercial areas such as ecology.

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Brownian and quantum motion

A recent demonstration that a classical problem in brownian motion belies Dirac's relativistic equations of quantum mechanics is a reminder of many things that students should be taught.

THE connection between brownian motion and quantum mechanics should be better known, at least to those brought up in the days before the full implications of Feynman's reformulation of quantum mechanics (in 1948) were fully appreciated, perhaps 15 years ago. That will be people's reaction to the neat argument, now published by B. Gaveau *et al.* (*Phys. Rev. Lett.* **53**, 419; 1984) which sets out to provide a demonstration that Dirac's relativistic wave equation can be derived from a consideration of a simple classical problem in brownian motion. Others will however note that the authors include Mark Kac, now at the University of Southern California, Los Angeles, who first derived the Schrödinger equation from a brownian motion problem in 1959, as well as T. Jacobson (University of Texas, Austin) and L.S. Schulman (Technion, Haifa), who have more recently made important contributions to similar problems.

The starting point for the argument is simple but evocative. Consider a particle moving in one dimension only at some fixed velocity, and let it suffer reversals of direction at random intervals, themselves distributed statistically according to some suitable law, most simply a Poisson distribution. Practitioners in brownian motion problems will readily write down expressions for the probability density (per unit length and per unit time) that the particle is at some place along the line at a certain time, and moving in one direction or the other. Most simply, there is a different function for each direction. The next step is to relate these probability densities to each other by taking account of the conservation in number of the particles moving along the line. The outcome is a set of differential equations which must be satisfied by the two probability densities. (In the sequel, these correspond to the two states of chirality or handedness which alone are accessible to relativistic Dirac particles in one dimension.) What the authors assert (and then prove) is that these equations are in an appropriate sense equivalent to Dirac's equation in one dimension. They then show that the same technique can be extended to the full relativistic problem (with three space dimensions).

The origin of this problem is intriguing, for it seems first to have appeared as an exercise for students in the elegant textbook *Quantum Mechanics and Path*

Integrals, by R. P. Feynman and A.R. Hibbs, published in 1965. There, users of the book are invited to calculate the evolution in time of a system consisting of a particle moving with the velocity of light in a single dimension, and suffering random reversals of direction. To Feynman and Hibbs, this would merely have been a test of what students had been told in the first thirty pages of the book. To Kac and company, the challenge has been to demonstrate that the connection between quantum mechanics and brownian motion that applies in non-relativistic mechanics also applies to Dirac's equation. That they succeed will not surprise those already in the know.

For the rest of us, there are several missing elements of which the least excusable is Feynman's use of the concept of the path integral. That, of course, is also familiar from classical mechanics. Since de Maupertuis and the principle of least action in the eighteenth century, it has been understood that the path that will actually be followed, and which is thus that dictated by the laws of motion, will be that for which the action (force multiplied by time) is least. For simple systems, forces can be calculated as a function of time from the function of the system called the lagrangian, essentially the difference between the kinetic and potential energies. Given initial and final states, and starting and finishing times, it is then possible to calculate (in principle at least) the action for all possible paths and to choose that for which the action is least.

The difference between this and quantum mechanics is that there is no unique path for the evolution of a system but that all possible paths must be counted. As Bohr and his disciples were always telling us, a photon confronted with two slits in a screen cannot be said to use one slit or the other, but both.

Each path is relevant; the only meaningful question to ask about the outcome is not where the particle finishes up, but the probability that it should finish up here or there and, to that end, that it is necessary to add together probability amplitudes (one for each possible path), taking account of plus and minus signs, and of imaginaries as well as real parts, before calculating the probability (by squaring the amplitude). The essence of Feynman's quantum mechanics is a powerful prescription for calculating the evolution of the amplitude for each

possible path. Inevitably, the outcome is a line integral. Although the integrals are not the same as those appearing in classical mechanics, their relationship is plain to see. In passing, it is worth asking whether quantum mechanics would ever be presented to students in other terms, necessarily less direct, if it were not that so much energy had been invested in teaching them to solve differential equations.

The connection with brownian motion is harder to discern. The formal resemblance with quantum mechanics is simple enough. Because the process is statistical, the most one can hope to do is to calculate the probability that a particle starting from one place on the line will finish up at another. Thus, if a particle is to start at one place and finish at another after a specified length of time, there is a large (even infinite) number of paths which it may traverse in doing so. The first to recognize this deeper relationship with Feynman's quantum mechanics — the existence of a multitude of paths — seems to have been K. Ito, working on brownian motion in 1951.

In reality a connection between the quantum mechanics of brownian motion gets even more specific. Put briefly, while the probability amplitudes in the brownian motion problem are all real and positive (and are thus simply additive), those which conspire in the calculation of Feynman's propagation functions are not. The quantum version of the calculations can be turned into the other version simply by replacing i (the square root of minus 1) by minus 1 whenever it occurs. The similarity is even more striking when one sees it demonstrated that a specific path in Feynman's formalism can be boiled down to calculating the chances that a brownian particle makes its way along the same such trajectory by random process.

The implication is simply grasped. The propagation of the probability amplitude along an arbitrarily chosen trajectory is indeed to be likened to brownian motion. One of the quirks of that process, the fact that the average displacement of a particle is proportional to the square root of the time elapsed, accounts for some of the computational complexity of Feynman's formalism. Taken together, the whole box of tricks makes it intelligible that there should be a correspondence between the theory of quantum fields and that of phase transitions on a lattice. This is yet another part of the case for changing tack on teaching.

John Maddox

Oceanography

Windscale effluent as a tracer for continental shelf circulation

from J. T. F. Zimmerman

CONCERN about the radioactive effluent from plants reprocessing nuclear fuel, primarily Sellafield (formerly Windscale) in Cumbria, UK, and to a lesser extent Cape la Hague near Cherbourg, France, has led to regular surveying, over more than a decade, of the radioactivity of the west European continental shelf waters, particularly by the Fisheries Research Laboratory in Lowestoft, UK, and by the Deutsche Hydrographische Institut in Hamburg. The results of the survey are not simply a means of monitoring a pollutant, but can be put to scientific use. As the flushing time scale of the Irish Sea–North Sea–English Channel system is of the order of several years, the outfall should by now have spread over the entire area. Tracing the fate of the radionuclides should therefore provide a method of testing hypotheses about the general circulation of these shelf seas. In a recent paper, D. Prandle of Bidston Observatory, Merseyside, UK, uses the radionuclide data to provide a numerical model of the water circulation of the area¹.

The radionuclide caesium-137 is an ideal conservative trace because its half life of about 30 years is long compared to the transport time scales on the shelf and because, being highly soluble, it is not easily removed from the water column by sedimentation. ¹³⁷Cs does not occur in nature. By the time the discharges from the reprocessing plants started, however, nuclear bomb tests had already created a background level in the Atlantic Ocean of 0.14 pCi l⁻¹, rising to 0.4 pCi l⁻¹ in the North Sea². The latter level has now been increased to 1–5 pCi l⁻¹, though this is still well below the permissible limit of 900

pCi l⁻¹ set by the International Atomic Energy Agency³.

What can be learned from the spread of ¹³⁷Cs over the contaminated shelf and even beyond that in the arctic waters of the North Atlantic Ocean? Early surveys^{2,4} already confirmed the traditional view⁵ of major transport routes of the water masses in the North Sea, Irish Sea and English Channel. Thus, generally, water from the Atlantic Ocean enters the English Channel, where it splits into two branches, one of which flows through the Straits of Dover, along the Dutch, German and Danish coasts to the Skagerrak area and finally is removed to the northern Atlantic Ocean by a northward current through the deeper Norwegian trench. A second branch splits off between Cornwall and Ireland, flows northwards through the Irish Sea, turns around Scotland, mixes with Atlantic water and then enters the North Sea near the Shetland–Orkney Islands. From there it flows southwards in a broad band on the western side of the North Sea to about 53°N, the latitude of the Wash, where it finally joins the flow from the Straits of Dover.

The distribution of radioactive effluent shows^{2,4} that the discharge from Windscale enters the North Sea through its northern entrance and gradually spreads over all of its area before being flushed to the northern Atlantic Ocean, whereas that from Cape la Hague primarily influences the eastern side of the North Sea basin.

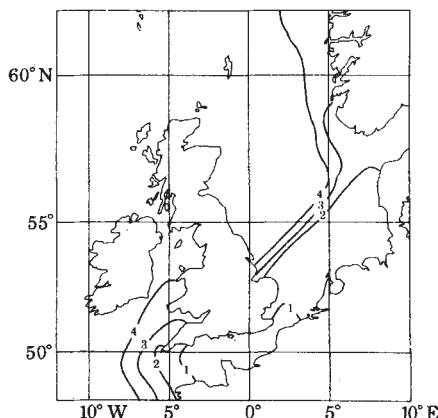
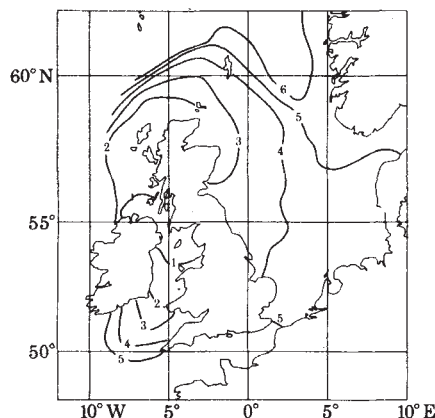
What are the velocities along these pathways? What is the dilution rate of a patch that follows a specific transport route and what kind of mechanism drives circulation and diffusion? Some of these

questions could be tentatively answered by looking at the evolution with time of the ¹³⁷Cs distribution pattern^{2,4} or by using simple box models for subregions of the shelf, particularly the North Channel and Irish Sea^{6,8}. In a much more ambitious use of the ¹³⁷Cs data, Prandle has now employed the recorded time evolution of the ¹³⁷Cs distribution over the continental shelf to calibrate a complete two-dimensional (vertically integrated) numerical model of its water circulation.

The model supports the 'traditional view' of the general circulation but also, more importantly, identifies the primary driving forces of the water movement and their relative strength. Thus, Prandle shows that windstress over the area, which is, on average, southwesterly in direction, sets the water on its course and that this circulation is reinforced and its speed increased by 10–50 per cent, by tidal rectification. Together, these driving forces create a more or less steady current with velocities of 1–6 cm s⁻¹, giving annual displacements of 500–1,000 km along the transport routes described above. This gives a transit time of 5 years for the Cornwall–Skagerrak route that passes the Irish Sea and recirculates into the North Sea and about half that time for the 'direct' route through the Straits of Dover.

Advection by currents is only half of the story, however. The tracer is also diffused by turbulent eddies, which cannot be resolved by a model. Turbulent diffusion coefficients have, therefore, to be introduced and it is here that the observed ¹³⁷Cs distribution can be used for calibration. The best way to parametrize turbulent diffusion coefficients in these areas is controversial. Prandle prefers to express them in the tidal current velocities, probably the safest choice. Calibration of the model with the aid of ¹³⁷Cs data gives values for the diffusion coefficients ranging from 50 m² s⁻¹ for the quiescent regions of the central North Sea to 600 m² s⁻¹ for the 'turbulent' Irish Sea. Thus, advection dominates diffusion over much of the North Sea area, whereas in the Irish Sea and English Channel the two transport processes are equally important.

Once supplied with diffusion coefficients, Prandle's model is able to predict a variety of time scales for the flushing of the shelf waters and the discharges from Windscale and Cape la Hague. It predicts that effluent from Windscale takes about 4 years to travel around the North Sea before entering the Atlantic Ocean through the Norwegian trench. The calculated bimodal distribution of transit times for Windscale, however, suggests that after 1 year about one-third of the discharge has escaped to the Atlantic Ocean by mixing during its passage through the waters around northern Scotland. The discharge from Cape la Hague — which is an order of magnitude less than that from Windscale — needs about 2 years to reach the Skagerrak via the



'Age' distribution (in years), as calculated by Prandle¹, for the effluent of Windscale (left) and Cape la Hague (right), where the 'age' of a constituent of a watermass is the average time it needs to travel from its discharge position to another arbitrary position in the area of consideration.

eastern North Sea. These values are of course related to the *e*-folding time for flushing of separate basins by the fluxes through their boundaries. For the North Sea and Irish Sea as a whole, Prandle's estimates for these scales are 530 and 328 days respectively.

For various subregions of the North Sea it is tempting to compare Prandle's estimates of the flushing time scale with those reported last year⁹ by a working group of the International Council for the Exploration of the Sea (ICES) led by L. Otto from KNMI, de Bilt, Netherlands. There are notable discrepancies: ICES observations suggest much more stagnant water masses along the east coast of England and in the centre of the North Sea gyre and more rapid flushing in the deep Norwegian trench than are predicted by Prandle's model (and others). Clearly the details of the circulation pattern are still open to question; nonetheless, Prandle's tuning of a circulation model with a suitable tracer has improved our understanding of the west-European shelf.

What happens to the radioactive effluent once it has left the shelf? At a recent symposium on 'Contaminant fluxes through the coastal zone' held in Nantes in May, Dahlgard and co-workers from the Riso National Laboratory, Denmark, and the University of Lund, Sweden, presented an updated version¹⁰ of their earlier conclusions¹¹ that the Windscale effluent reaches the East Greenland Current in about 6–8 years, probably after being transported northwards along the Norwegian coast and across the Atlantic south of Spitsbergen. Although crude, this picture fits in very well with Dahlgard's results for the North Sea. These predict transport times and a dilution rate of the Windscale effluent as follows. If its concentration is set at 1,000 in the northern Irish Sea where the material stays for 1 year, say, it will take about 3 years to cross the North Sea during which time its concentration drops to 100. The passage along the Norwegian coast takes another year, with a dilution of another 50 per cent, and by the time the material reaches the waters near Greenland, within 2–4 years, its concentration will be about a thousandth of its original value. □

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Molecular biology

Excision of introns in lariat form

from Charles Weissmann

SEVEN years after the discovery that the protein-coding sequences of most genes are disrupted by introns, an astonishing pathway has been revealed for the splicing (that is, excision) of introns from gene transcripts. In short, both Sharp's group^{1,2} and Maniatis, Green and their colleagues³ have shown that, contrary to expectation, the introns that are excised in the production of messenger RNA (mRNA) from its precursors (ultimately the gene transcripts) are recovered as lariat-like structures rather than as linear or circular RNA. The lariats are formed by attachment of one end (5') of the intron to a site close to the other (3').

This discovery has followed the recent development of cell-free systems for the splicing of preformed mRNA precursors^{4–6}. As a substrate for their cell-free system Sharp and his colleagues used a ³²P-labelled RNA, prepared by the Manley system⁷, that consisted of the major adenovirus 'late' exons (L1 and L2)

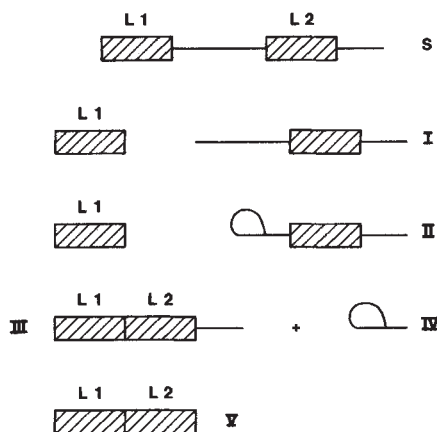


Fig.1 Structures of the products (I–V) of cell-free splicing of a substrate (S) RNA composed of two exons (L1 and L2) and two introns. IV is the excised intron in lariat form.

separated by an abbreviated intron and with a short second intron following L2. This substrate is schematized as 'S' in Fig.1. Earlier this year they had reported⁴ that prolonged incubation of the substrate with a HeLa cell extract in the presence of ATP and Mg²⁺ gave rise to four major products (now identified as structures II to V in Fig. 1), three of which accumulated, while one (II) appeared transiently with the kinetics expected of an intermediate. The sequences of these products were investigated by fingerprint analyses and hybridization experiments. III was one of the expected splice products, with L1 joined to L2 and followed by the second intron; V resembled III but lacked most or all of the second intron (it is believed to be a degradation product of III).

As judged by its oligonucleotide map, IV

corresponded to the intron, but it showed two unexpected features. First, it had an abnormally low electrophoretic mobility for its molecular weight and when subjected to limited RNase T₁ digestion gave rise to a discrete product with a lower mobility. Second, its complete digestion by RNase T₁ gave rise to a modified oligonucleotide not present in the precursor RNA. The putative intermediate II contained the sequences of the first intron, the second exon and the second intron; it too showed an abnormal mobility and was digested by RNase T₁ to the same modified oligonucleotide as IV. The modified oligonucleotide resisted complete digestion by nuclease P₁, suggesting to Grabowski *et al.* that it might contain a branchpoint of the kind previously found in total polyadenylated nuclear RNA by Wallace and Edmonds⁸, who, interestingly, suggested that the branched RNA species could be splicing intermediates. To explain its properties, Grabowski *et al.* suggested that IV contained a circular moiety.

The new papers by Padgett *et al.*² and Ruskin *et al.*³ show beyond reasonable doubt that product IV and putative intermediate II have the lariat-like structures indicated in Fig. 1. The additional evidence offered by Padgett *et al.* for the structures of IV and II is as follows: the intron-derived product IV has a free non-phosphorylated 3' terminus; complete digestion of the modified oligonucleotide with RNase T₂ gives rise to a nuclease-resistant oligonucleotide, namely 2', 3'-ADP linked to two nucleotides (presumed to be 3'-UMP and 3'-GMP) by 3'-5' and 2'-5' phosphodiester bonds respectively, as shown in Fig. 2; and in both II and IV, the 5'-terminal region of the intron is linked to the modified oligonucleotide, which contains the sequence running from nucleotides 19 to 32 upstream of the 3' splice site.

Ruskin *et al.*, employing a technically distinct approach, used as their substrate a truncated β -globin RNA, prepared by the SP6 RNA polymerase system⁹ and consisting of the first exon, the small intron and part of the second exon, to which was added a 5' cap using vaccinia virus guanylyltransferase. With an extract of cell nuclei as the source of splicing activity, the species corresponding to I (mentioned in passing by Sharp and his colleagues) and II were produced during the early stages of the reaction, while those corresponding to III and IV accumulated later. Ruskin *et al.* characterized the lariat-like structures on the following basis: first, reverse transcriptase elongation of a primer complementary to a segment of the second exon was blocked about 37 nucleotides

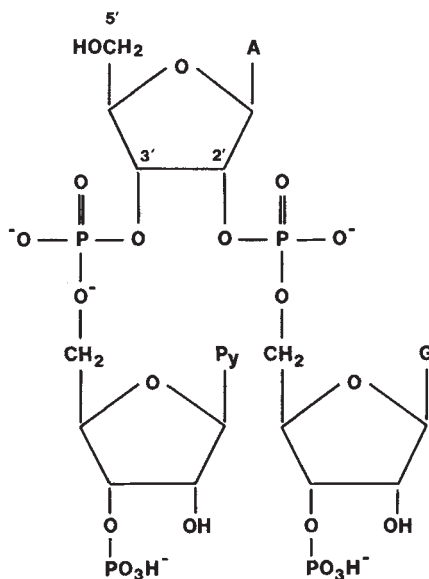


Fig. 2 Digestion product of the modified oligonucleotide formed at the attachment site of the lariat-like structure of an excised intron.

upstream of the 3' splice site, while a primer complementary to a segment of the first intron upstream of the block was elongated to the 5' end of the intron; second, when (and only when) an oligodeoxynucleotide complementary to the intron was hybridized to the globin counterpart of IV, RNase H treatment led to a strong shift in electrophoretic mobility but did not give rise to a second RNA fragment, showing that cleavage must have occurred within a circular entity (an analogous result was obtained for intermediate II); third, a nuclease-resistant branched oligonucleotide with the structure shown in Fig. 2 (where Py = cytosine) was characterized, and the branchpoint located at the thirty-seventh nucleotide upstream of the 3' splice site.

What does all this mean? It is quite likely that products I and II represent intermediates on the way to the final product. The suggestion is that splicing involves cleavage at the 5' splice site of the intron, yielding a 5'-phosphorylated guanosine residue at the 5' terminus of the intron which then becomes joined to the 2' hydroxyl group of the branch acceptor nucleotide (an adenosine residue) within the intron. The reaction may be concerted in the sense that the attack of the branch acceptor site on the 5' terminus of the intron cleaves off the first exon while forming the lariat. In the next step, the cleaved 3' end of the first exon, which is presumed to remain bound to a putative splicing complex, attacks the phosphate between the last (3') nucleotide of the first intron and the first (5') nucleotide of the second exon, releasing the intron as a lariat while joining the two exons.

Several questions are raised by the new findings. Are there further intermediates? As an incubation of 15 min in the presence of ATP is required before intermediates I

and II are first detected, it is likely that some essential, as yet uncharacterized, event must occur before the cleavage at the 5' splice site. This could, for example, be either the formation of a nucleoprotein particle in which the precursor RNA would assume the conformation necessary for splicing, or an ATP-dependent activation of the branchpoint acceptor.

Is the branch acceptor nucleotide (usually an adenosine residue⁸) embedded in a highly specific sequence? This is unlikely in the case of eukaryotic introns, because, as shown by Wieringa *et al.*¹⁰, deletions or substitutions that remove all but 15 nucleotides adjoining the 3' splice site of the large β -globin intron do not impair splicing. In the case of the intron in yeast nuclear genes, however, an internal sequence (TACTAAC) is essential for splicing¹¹ and Pikielny *et al.*¹² have in fact mapped what may be a reverse transcription stop in this region. Ruskin *et al.* note that all β -globin introns contain a 'consensus sequence', of which the TACTAAC sequence is a subset, located in the region between 19 and 37 nucleotides upstream of the 3' splice site. The consensus sequence is: Py-X-Py-T-Pu-A-Py (the underlined A represents the branchpoint in β -globin). The sequence in which the branchpoint identified by Padgett *et al.* for adenovirus is embedded conforms to this consensus sequence except that the fifth residue is a pyrimidine (Py) rather than a purine (Pu).

Since at least some of the intron deletions examined by Wieringa *et al.* do

not contain a reasonable facsimile of the consensus sequence but are nonetheless efficiently spliced, it would seem that even if branch formation preferentially occurs within the consensus sequence, it may occur elsewhere if a consensus sequence is lacking; moreover, it remains to be proved that branch formation is essential for splicing.

Why does the 5' end of the intron become joined to an internal nucleotide? It is striking that the introns of *Tetrahymena* ribosomal RNA and of yeast mitochondrial precursor mRNAs are released in circular form. Perhaps, as Ruskin *et al.* suggest, the initial cleavage may be reversible, and branch or circle formation are required to sequester the 5' intron definitively, allowing the next reaction step to occur. Thus, although splicing in higher eukaryotes differs in many important respects from that in lower eukaryotes, they may have some mechanistic principles in common. □

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Cystic fibrosis

In search of the gene

from Veronica van Heyningen

SADLY, the future of the 10th International Cystic Fibrosis Congress seems assured. The enigma of the primary gene defect in cystic fibrosis — the most common genetic disease in Caucasian populations (1 in 2,000 births) — has not yielded to the wide-ranging biochemical, physiological and molecular genetic approaches described at the 9th Congress*.

Cystic fibrosis is a disease of the exocrine glands which is inherited in an autosomal recessive fashion. It is characterized by the production of abnormally thick mucus in many organs, resulting typically in chronic obstructive lung disease; by pancreatic insufficiency often leading to malabsorption; and by abnormally high levels of sodium and chloride in sweat. This last feature is the basis for definitive diagnosis.

Despite the steadily improving prospects for afflicted individuals, the social implications of the disease are still serious, as was reflected in a study of parental

attitudes in Californian cystic fibrosis families (M. Kaback *et al.*, University of California, Los Angeles). In 65 per cent of the 183 families studied, the affected child was the last born to the couple and more than a quarter of the couples resorted to surgical sterilization to avoid the possibility of having further offspring with the disease. Reliable prenatal diagnosis would clearly be welcome to such families.

Brock (*Lancet* **ii**, 941; 1983) and colleagues have found reduced levels of intestinal microvillar enzymes in amniotic fluid if an affected fetus is being carried in a pregnancy 'at risk' (previous cystic fibrosis child born to the parents). The reduced levels are thought to be secondary to the impaired passage of meconium in the cystic fibrosis fetus. Following the failure of an earlier prospective trial for reduced amniotic fluid protease activity (Nadler, H.L. & Walsh, M.M.J. *Pediatrics* **66**, 690; 1980) great caution is being exercised in confirming and extending these findings. Nonetheless, groups in Rotterdam (W.J. Kleijer *et al.*, Erasmus University), Paris

*9th International Cystic Fibrosis Congress, Brighton, 9-15 June 1984. The proceedings will be published this year (*Cystic Fibrosis: Horizons*; ed. Lawson, D., Wiley, Chichester; 1984).

(A. Boué *et al.*, Inserm U73) and Glasgow (D.A. Aitken *et al.*, University of Glasgow) are successfully applying microvillar enzyme estimations prospectively. Validation of this approach to prenatal diagnosis would be speeded up if definitive diagnosis of cystic fibrosis were possible in the fetuses declared abnormal and aborted on parental request. Confirmation of normal outcome in pregnancies carried to term is also difficult because there is considerable variability in severity and age of onset of overt disease, although neonatal sweat testing and immune-reactive trypsin measurement of newborns may help.

While there is every indication that prenatal diagnosis of this kind will help prevent the birth of a second affected child in families at risk, the assays are based on what is almost certainly a secondary phenomenon and the race to identify the basic gene defect is still on. Considerable excitement has been generated by recent work on isolated sweat glands (Quinton, P.M. *Nature* **301**, 421; 1983) and on nasal epithelium (M.R. Knowles, R.C. Boucher *et al.*, University of North Carolina). In both cases, there appears to be a defect in ion transport in affected individuals. Thus, raised potential differences across the apical membrane of epithelial cells suggest reduced chloride permeability, and this is accompanied by reduced responsiveness to β -adrenergic stimulus by isoproterenol. The possibility that the abnormal β -adrenergic responsiveness and secretion in cystic fibrosis results from a defective calcium-dependent regulatory protein was suggested by several workers, for a variety of tissues (R.M. Case, University of Manchester; G. Cabrini *et al.*, Centro Fibrosi Cistica, Verona; M. McPherson *et al.*, University of Cardiff). One of the few groups to study heterozygote as well as homozygote red cells observes a calcium transport defect in both (C.A. Seymour and J.A. Davis, University of Cambridge).

As long as the basic gene defect remains unidentified, the choice of tissue for study will be arbitrary and often made on the basis of availability. The ability to culture sweat gland epithelia (P.S. Pedersen, Rigshospitale, Copenhagen) may prove a useful stimulus in this field. Meanwhile, considerable effort is being made to set up reliable heterozygote detection using serum samples (Manson, J.C. & Brock, D.J.H. *Lancet* **i**, 330; 1980) as this would help in the search for the defective metabolic pathway and ultimately the primary mutant gene locus (A. Jamieson *et al.*, University of Glasgow; V. van Heyningen *et al.*, MRC Clinical and Population Cytogenetics Unit, Edinburgh; O. Guy-Crotte *et al.*, Inserm U31, Marseille). Various teams (R. Williamson *et al.*, St Mary's Hospital Medical School, London; K.W. Klinger, Case Western Reserve University, Cleveland; Lap-Chee Tsui *et al.*, Hospital for Sick Children, Toronto; and R.H. Schwartz *et al.*, University of Rochester)

are setting up DNA banks or permanent cell lines from cystic fibrosis families with two or more affected children.

The only available method of searching for the cystic fibrosis gene is linkage analysis by exclusion mapping using polymorphic DNA probes that have been mapped to a known chromosome region. In this way, using samples only from affected individuals and obligate heterozygotes, a number of chromosome regions have been found not to be linked to cystic fibrosis. Just before the congress took place, there was excitement about the pos-

sibility of assigning the cystic fibrosis gene to the tip of the long arm of chromosome 13 on the basis of an informative family with a segregating translocation (Edwards J.H., Jonasson J.A. & Black N.L. *Lancet* **i**, 1020; 1984), but this now seems unlikely. Nonetheless, an air of optimism pervaded the conference because the introduction of new methods such as electrophysiology and gene cloning brings some promise of breakthrough. □

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Cellulose synthesis

Another brick in the wall

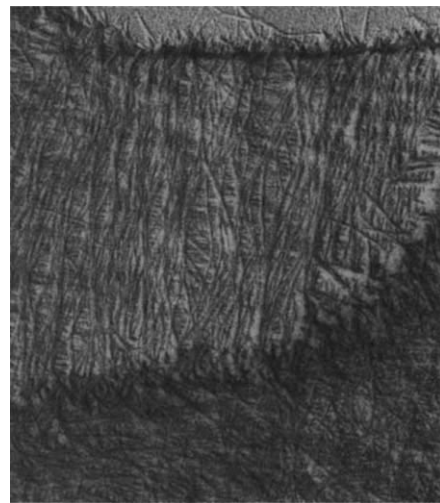
from Keith Roberts

It is estimated that almost 2×10^{12} tonnes of carbon are wrapped up in organic molecules on the landmasses of this planet. The most abundant of these molecules, accounting for just over one-third of the dry weight of plants, is cellulose. Indeed, there is so much of it that cellulose forms the major buffer for the levels of carbon dioxide in the atmosphere. For a macromolecule of such abundance and economic importance (wood, paper, cotton), it is really rather astonishing that we still do not know in detail how it is made (Colvin, J.R. in *The Biochemistry of Plants*, ed. Preiss, J., 543, Academic, New York; 1980). What is clear is that higher plants have a remarkably complex and delicate machinery for synthesizing the basic building blocks of cellulose, the glucan chains, and for ordering their crystallization and orientation in the cell wall. The difficulty in trying to dissect the complexity of this machinery has been in obtaining an *in vitro* system that will make identifiable cellulose (Delmer, D.P. *Adv. Carbohydr. Chem. Biochem.* **41**, 105; 1983). Until now, despite many false alarms in the literature, no one has managed to do this. However, a report in this issue of *Nature* at last holds out some promise by describing the production of cellulose *in vitro* using a membrane preparation from a higher plant.

Cellulose is a (1 $\rightarrow$ 4)- β -D-glucan, consisting of many long parallel polysaccharide chains held together by inter- and intramolecular hydrogen bonds to form microfibrils, typically 10–40 nm in diameter. The microfibrils are extremely insoluble and form the high-tensile-strength skeletal framework of the plant cell wall (see the figure). The consensus of opinion is that the enzyme or enzyme complex that produces cellulose is an integral protein(s) of the plasma membrane. Most efforts to achieve cellulose synthesis *in vitro* have therefore started with isolated membrane preparations, usually either from rapidly elongating tissue such as the hypocotyl, or from cells laying down large

amounts of cellulose, such as those that make cotton fibres.

Why has none of the previous attempts to mimic *in vivo* cellulose synthesis with *in vitro* membrane preparations been completely successful? The answer, at least in part, lies in the complexity of the process itself and in the structural constraints on the system which have to be preserved *in vitro*. Initially, even the precursors that the plant uses to make cellulose were the



500 nm

The complex texture of cellulose microfibrils *in vivo* is revealed in this metal-shadowed specimen of an extracted primary cell wall. The specimen comes from a root hair cell of an onion. (Micrograph by B. Wells.)

subject of dispute, with opinion slowly shifting from GDP-glucose to UDP-glucose. Intact cells take up and use exogenous UDP-glucose very poorly, the cellulose synthase complex accepting UDP-glucose only on its cytoplasmic face. Somehow UDP-glucose has to gain access to the inner face of the membrane and, in general, cell breakage leads to inactivation of the synthetic complex. There has also been disagreement about the role, if any, of various intermediates. Both lipid-linked intermediates and glycopro-

tein intermediates have been invoked at various times, but neither has been conclusively shown to be involved. Uncertainty also prevails over the requirements for Ca^{2+} and Mg^{2+} , GTP and various other soluble cofactors. The biggest stumbling block and the greatest puzzle have been that *in vitro* systems used so far have invariably been able to incorporate glucose into high-molecular-weight material, but the product always seems predominantly to be (1 $\rightarrow$ 3)- β -D-glucan (callose) and not (1 $\rightarrow$ 4)- β -D-glucan (cellulose). The reasons for this are unclear but it was suggested at two recent meetings* that cell damage or wounding stimulates callose production at the expense of cellulose, possibly because of altered Ca^{2+} access to each side of the membrane. It was even suggested by D. Delmer (ARCO, California) at the Fribourg meeting, and by D.H. Northcote (University of Cambridge) at the Glasgow meeting, that the same enzyme complex, regulated by Ca^{2+} , may be involved in the synthesis of both polymers.

Clearly, the complexity of the synthetic system is great and a model system is required. In this case, as in so many branches of cell biology, the model system is a bacterium, *Acetobacter xylinum*, which produces a cellulosic pellicle in liquid culture. Two years ago, a collaboration between D. Delmer and M. Benziman's group in Israel resulted in high rates of *in vitro* synthesis of cellulose using a stabilized membrane fraction from this bacterium (Aloni Y. *et al. Proc. natn. Acad. Sci. U.S.A.* 79, 6448; 1982). Success appeared to depend crucially on the presence of polyethylene glycol 4000 (PEG) which stabilizes sealed membrane vesicles and aggregates proteins.

Now, in another attempt with higher plants, T. Callaghan, working with Benziman, has elegantly applied the same basic methodology to membrane fractions from mung bean hypocotyls, apparently with remarkable success (see page 165 of this issue). Using a PEG-stabilized preparation, they are able to achieve rates of cellulose synthesis that approach those found *in vivo*. Notably, no detectable (1 $\rightarrow$ 3)- β -D-glucan is formed, Ca^{2+} and Mg^{2+} are both essential, no intermediates are formed and cellobiose and another low-molecular-weight soluble factor increase the rate of synthesis. Although the crucial final X-ray demonstration of authentic cellulose has not been done, the notoriously difficult chemical characterization of the product appears convincing.

At present it is hard to identify the reasons for this success where previous attempts had failed. One reason put forward is that because the system is very complex, it makes sense to tamper with it as little as possible; thus high-speed spins and any attempt to 'clean up' the membrane

fraction have been avoided. This, together with the use of stabilizing PEG and the correct choice of cofactors, may have tipped the scales in Callaghan's and Benziman's favour. It will be interesting to see whether the new methodology is repeatable and whether it can be used with other plant material.

If it does prove to be a viable system for the *in vitro* synthesis of cellulose and not, for example, a way simply of making the glucan backbone of hemicellulose molecules, the door is opened for exploring a whole range of extremely important issues in higher-plant cell biology. As well as being able to investigate the factors that control the width and length of cellulose microfibrils, the separation or otherwise of the synthesis and crystallization processes and the true relationship between callose and cellulose synthesis, it should also be possible to attack one particularly thorny critical problem. For many years it has been suggested that particular plasma

membrane complexes seen in the electron microscope correspond to cellulose synthase assemblies (Giddings, T.H. *et al. J. Cell Biol.* 84, 327; 1980). An extension of this idea, which still has no strong experimental backing, is that the complexes move through the plane of the membrane, spinning out microfibrils into the extracellular matrix as they go. Elements of the plant cytoskeleton, the cortical microtubule array, are thought to define membrane channels in which the complexes are free to move, and may therefore control the orientation of cell-wall microfibril deposition. As wall texture is fundamental to organized cell expansion, this phenomenon is at the heart of many fundamental problems of plant cell growth and differentiation. It looks as though at least one possible avenue for attacking them has been opened up. □

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Sex chromosome evolution

Sharing outside of pairing

from R.P. Erickson and P.N. Goodfellow

THE human X and Y chromosomes are very different in appearance and genetic content and yet are believed to be derived from an ancestral pair of chromosomes differing only at a single sex-determining locus. Several models for the evolution of mammalian sex chromosomes have been proposed to explain sex chromosomal differentiation but experimental evidence to decide between the models has been lacking. Analysis of cloned DNA sequences from the human sex chromosomes, particularly the Y chromosome, should eventually solve the problem of how the X and Y chromosome have evolved and should provide clues to the mechanism of sex determination. This approach has been used by two groups who report their results in *Nature* this week and next.

Genes determining testicular and ovarian development predate fixed chromosomal sex determination in evolution. One can imagine that two nonhomologous chromosomes separately accumulated genes for sexual differentiation, genes for male development on an ancestral Y and genes for female development on a non-homologous ancestral X. However, evolution of sex chromosomes from a homologous pair appears frequently in vertebrate evolution and is the usually accepted mechanism.

Given a pair of homologues, one with male-determining genes, two theories have been proposed to explain the evolution of sex chromosome differentiation. The classic model of sex chromosome evolution from homologues places cross-over suppression between the ancestral pair as the primary event with a slow accumulation

of differences by mutation¹. Later, X-chromosome inactivation occurs to maintain the gene dosage for genes now found only on the X chromosome. An alternative theory puts chromosomal inactivation of the non-sex-determining portion of the sex chromosomes first², solving the male/female sex-chromosomal dosage problem at the start. The selective force hypothesized in the classic model is the need to keep a block of sex differentiation genes intact. The selective force in the alternative model is the still obscure advantage of chromosomal sex determination. Both models assume continued crossing-over between homologous Xs and, thus, explain the observation that while the X chromosome has retained many structural genes apparently unconnected with the process of sexual differentiation, the much smaller Y chromosome seems to have lost all but a small number of genes directly concerned with sex determination and male gametogenesis.

There are two major limitations on sex chromosome differentiation. First, sex chromosome pairing must occur at meiosis for normal chromosomal disjunction. Chromosomal pairing is thought to depend on sequence similarity and this implies that the X and Y chromosomes should show sequence homology in the X-Y pairing region. Light microscopic studies of human meiosis have shown that the pairing region includes the terminal third of the X-chromosome short arm and most of the Y-chromosome short arm³. Second, the sex-determining region must not be disrupted by recombination with the X

Third cell wall meeting, 26-29 March, Fribourg, Switzerland, and SEB Seminar Series symposium 'Plant cell walls', 10-11 July, Glasgow.

chromosome. The consequences of transfer of sex-determining genes from the Y to the X chromosome can be seen in the generation of sterile XX males by the transfer of the *Sxr* fragment in the mouse^{4,5}. A similar event has been demonstrated in some XX human males who have inherited Y-derived sequences⁶. Correlation of clinical and cytogenetic findings in patients with rearranged Y chromosomes has localized the sex-determining region to around the centromere of the Y chromosome, well outside the pairing region⁷. Thus, it would be predicted that homologous sequences shared by the X and Y chromosomes would be restricted to the X-Y pairing region. This prediction is compatible with the localizations of the only expressed gene known to be homologous between the X and Y chromosomes⁸. Studies with cloned sequences derived from the Y chromosome, however, have found considerable sequence homology between the X and Y chromosomes outside the pairing region.

Early studies of human Y-chromosomal DNA emphasized the large amount of relatively Y-specific repeat elements (reviewed in ref.9). The cloning of Y-chromosomal DNA in cosmid vectors, however, has led to the isolation of unique-sequence sub-clones¹⁰⁻¹³, many, but not all, of which also hybridize to the X chromosome. Furthermore, several clones from X-chromosome libraries have been found to hybridize with the Y^{13,14}. Many of the X and Y shared sequences have been mapped to the short arm of the Y and the long arm of the X¹¹⁻¹⁵: the homology does not fit the pairing seen at meiosis. The simple prediction is also not supported by the two new studies reported in *Nature*. One of the first sequences shown to be shared by the X and Y chromosomes was isolated as a random fragment from a total human genomic library¹⁶. On page 119 of this issue, Page *et al.* show that the homology region extends for at least 36 kilobases (kb) and maps to the short arm of the Y chromosome and the long arm of the X chromosome (Xq13-Xq21), well outside the X-Y pairing region¹⁷. Careful restriction mapping suggests that the X and

Y sequences are at least 98 per cent homologous. An even greater degree of homology has been found by Cooke and Rappold (next week's *Nature*) for a sequence first isolated from a genomic library made from flow-sorted Y chromosomes¹⁸. Direct sequencing of the X- and Y-derived clones shows no differences in over 1 kb. This DNA segment too maps to the long arm of the X chromosome, but it maps to the heterochromatic region of the Y chromosome, well outside the pairing region.

Page *et al.* have gone on to study the origin of their homologous sequence by investigating DNA from male and female primates¹⁷. They find that in chimpanzees, gorilla and orang-utan, the sequence is found only on the X chromosome. Genomic mobility may be a feature of many Y-chromosome sequences and has been documented previously both for the Y-specific repeats and unique sequences. The 3.4-kb repeat, which may constitute as much as 40 per cent of the human Y chromosome, may have an autosomal origin⁹. In this case, transfer of a few repeats followed by rapid expansion

mediated by unequal crossing-over or gene duplication (or both) could be envisaged. For other sequences found on the X, Y and autosomes, such as actin¹⁹ and argininosuccinase²⁰ pseudogenes, a mRNA origin is likely. It is not clear that such mechanisms can explain the transfer of the 36 kb segment described by Page *et al.*, nor the other X-Y homologous sequences reported to map outside the pairing region. At least one of these other sequences also spans greater than 20 kb (ref.13). The high degree of sequence conservation suggests that the X-Y exchange must have occurred relatively recently, perhaps as the result of double recombination events facilitated by normal X-Y pairing or as a function of an ancient pre-existing homology. It is also possible that a new mechanism analogous to gene conversion but occurring on a larger scale may be operating. □

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Embryonic development

Cell communication in early embryos

from Jonathan Slack

'LOW-RESISTANCE' junctions between cells in embryos and many adult tissues were discovered in the 1960s by their ability to pass electric current¹. Later it became clear that they were the same as the 'gap junctions' seen under the electron microscope as open channels connecting adjacent cells, and that they were capable of passing most substances of molecular weight less than about 1,000 (ref. 2). A popular view has been that gap junctions transmit the inductive signals that control regional specification in the early embryo. But although the junctions have been mapped in embryos of various types³⁻⁶, functional evidence for this interpretation has so far been lacking because there has been no method of blocking the junctions *in vivo* without unacceptable side effects. Now, in this week's *Nature*, Warner, Guthrie and Gilula describe a means of doing just this by injection of antibody against gap junction protein into early *Xenopus* embryos⁷. The authors are very cautious in their interpretation; nonetheless, the experiments provide the first direct evidence that gap junctions indeed have a role in early embryonic development.

Before these experiments could be done, it was necessary to know the normal disposition of junctions in the *Xenopus* embryo. Previous electrophysiological studies had shown that cells of the early embryo were coupled³, but the work was

done without reference to the positions of the cells studied. In the paper by Guthrie in this week's *Nature*⁸, each of the blastomeres in the animal hemisphere of the 32-cell stage has been injected with the fluorescent dye Lucifer Yellow and it is shown that dye transfer is much more frequent between the cells on the dorsal side than between those on the ventral side.

Warner *et al.* therefore selected a dorsal blastomere of the 8-cell stage for injection of antibody to gap junction protein, and then studied dye-coupling at the 32-cell stage between the progeny of the injected cell⁷. They find that the frequency of dye-coupling is substantially less than in untreated embryos and in most cases cells which fail to transfer dye are also uncoupled electrically. By contrast, injection with preimmune serum or an unrelated antibody is without effect.

At later developmental stages the injected embryos show substantial morphological defects in the region derived from the injected blastomere: the brain and eyes being absent or reduced. These defects do not arise from cell death, which would be very obvious in an embryo type which develops without increase in size. Rather, they appear to be due to a failure of neural induction since in normal development the neural tube is induced from dorsal ectoderm by the action of the underlying mesoderm. This might be because the tissue

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movements during gastrulation have been disturbed so that the disposition of ectoderm and mesoderm is abnormal; alternatively, it might be the result of a direct blockage of the transmission of the inducing signal. It should be possible to distinguish between these possibilities by co-injecting a lineage label⁹ with the antibody to enable movements of the affected cells to be followed.

A direct role for gap junctions as channels for the neural inducing signal is perhaps unlikely in view of the fact that at least the early steps of neural induction can occur in the absence of any cell contact¹⁰, implying that the active substances can diffuse across extracellular spaces. Nonetheless, neural induction shows considerable regional specificity, particular regions of the archenteron roof inducing corresponding regions of the nervous system¹¹,

so the whole process is probably more complex than the transmission of a single substance. Clearly, gap junctions are involved in some way but more work is needed on the unfashionable problem of the biochemistry of inducing factors before we can make a final assessment. □

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Geology

Geological problems of north-east Africa

from Robert Bowen

EARTH satellites are helping to uncover valuable data about the sub-surface topography of arid regions such as the western Sahara and the Mojave deserts. So much was clear from an international meeting* on the geography of north-east Africa earlier this year, in advance of the launch of the US NASA shuttle later this year of the second shuttle imaging radar (SIR-B) designed by the Jet Propulsion Laboratory (Pasadena).

The first SIR experiment in 1982 led to the still remarkable reports of 'radar river' deposits, described by J. McCauley and C. Breed for themselves and G. Schaber (U S G S, Flagstaff, Arizona). They occur as buried channels in the Western Desert of Egypt, where broad valleys and younger inset channels have been identified using radar responses from surface and sub-surface materials including caliche horizons in fluvial sediments. Such features have promoted a new understanding of the regional role of running water in shaping the hyperarid core of the Sahara, before the onset of the periodic Quaternary aridity, and have made it possible to understand fluvial environments including some containing 'Acheulian' artefacts of presumed late Pleistocene age, which have been located incidentally to stratigraphic investigations in backhoe trenches.

In an extension of the radar technology (R. Blom, JPL), igneous dykes beneath as much as 2 m of alluvium were detected in the Mojave Desert by the Seasat L-band (23.5 cm) synthetic aperture radar in 1978, when soil moisture probably fell below the

1 per cent critical microwave absorption level. Blom pointed out that the Mojave is a 'swamp' compared to the hyperarid eastern Sahara, and can be examined only when moisture content declines sufficiently. Other factors such as the roughness and the dihedral configuration of the dykes were significant because they helped in the generation of strong radar echoes. Clearly, conditions suitable for the detection of subsurface features may exist intermittently in places previously considered unsuitable, although the definitive interpretation of radar tracking image data may need subsequent field work.

There was some controversy at this meeting over Egyptian and Sudanese stratigraphy, a supracratonic arenaceous sequence extending through most of the Phanerozoic. Until recently, access has been difficult while the marginal interest of the petroleum companies has reduced further the number of geologists actively working in these countries. However, in the last couple of decades, progress has been made by attempts to clarify the so-called Nubian sandstone (B. Issawi, Egyptian Geological Survey; E. Klitzsch, Technical University of Berlin).

The disagreement derives from correlation of clastic beds, especially those of supposed Palaeozoic age. The stratigraphic position of fluvio-glacial deposits occurring both in Egypt and in Sudan, originally given as Ordovician by Klitzsch, has been traced laterally from Libya across to Saudi Arabia by U. Jux (University of Cologne). The situation is now unsatisfactory because Klitzsch (*J. Afr. Earth Sci.* 1, no.1, 17; 1983) re-assigned the

deposits to the Permo-Carboniferous ('karroo-type'), which implies an equivalence to the Dwyka glaciation of southern and south-central Africa and of course a totally new concept of Gondwana and its biotas. The pre-Carboniferous Palaeozoic column in southern Egypt is devoid of index fossils, so that the stratigraphy is to a large extent based on ichnofossils such as Cruziana and Bifurcites, the identity and reliability of which were disputed.

Proper correlation is presently impossible in this vast terrain which was previously considered to embody a 'pancake'-type geology but, because of epeirogenic movements of the Uweinat-Aswan geanticlinal arch, is now known to have been marked by cyclic transgression and regression events involving deposition and erosion (D. Bhattacharyya, Washington University; B. Issawi). Klitzsch takes the view that there was a change in the fluvial discharge direction in Egypt and Sudan, reversing an earlier north-south flow, but more data are needed to resolve this question.

Obtaining more detailed geological information is even more necessary in Sudan where, as R. Stern (University of Texas, Dallas) pointed out, sampling is hardly richer than on the Moon. He presented some interesting chronometric results supporting a model for the tectonic evolution of the Sudanese basement in the late Proterozoic.

Another very controversial subject is that of the aquifer systems of Libya and Egypt, where the occurrence of recharge and groundwater flow are in dispute. M.U. Ahmad (University of Ohio) presented a steady-state groundwater flow model for the southern Libyan basins which has been applied to test the existence of recharge through the past five thousand years. Ahmad believes this to have occurred, in which case the water problems of the region can be solved. The flow rate from Libya to Egypt is given as almost 4 million m³ daily, a figure with which Issawi strongly disagrees.

The meeting ended with a lively discussion of the Libyan glass enigma. J. Underwood Jr (Kansas State University) and R. Giegengack (University of Pennsylvania) cited various unacceptable explanations and an unpublished paper by U. Jux which deals with the differing origins of Libyan astroblemes and the glass. Underwood, Giegengack and Jux agree on a terrestrial origin, and the only viable model appears to be that suggested by Jux in 1983 — a diagenetic silcrete-type mechanism of formation under early Neogene semiarid conditions resulting from reaction between superficial waters containing carbon dioxide and alkaline highly-siliceous subterranean and groundwaters. □

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Gravitational interactions of cosmic strings

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Breaking a Yang–Mills symmetry at very high energy could lead to macroscopic structures in the vacuum called ‘strings’, which may be the cause of galaxy formation and clustering. Dissipative gravitational interactions of string loops can produce unusual astrophysical side effects. Such scenarios generally predict a stochastic background of gravitational radiation which should be observable using the millisecond pulsar 1937+21. Gravitational radiation recoil accelerates loops, but dynamical friction on ordinary matter tends to slow them down so they may be able to accrete matter. Internal oscillations of loops produce a time-varying shear perturbation which may lead to an observable heating of stellar or gaseous systems in their immediate neighbourhood.

THE general acceptance of Yang–Mills theories of fundamental interactions, and the associated idea of their intrinsic symmetries being ‘broken’ by an asymmetric vacuum, have led physicists to endow the vacuum with an ever-increasing number of degrees of freedom and intrinsic structural properties. The vacuum, as now envisaged, can sustain long-lived and even macroscopic structures. A vacuum in three-dimensional space can have three types of topologically stable ‘defects’—monopoles, strings, and domain walls, corresponding to zero-, one-, and two-dimensional surfaces in which the vacuum is ‘trapped’ in its high-energy symmetric state¹. Astrophysical effects of magnetic monopoles have attracted much attention recently because of their possible revolutionary implications for a ‘grand unified’ Yang–Mills theory and for cosmological models². The possibility of relic cosmic strings has also been studied, primarily with a view towards using them to produce the density fluctuations leading to galaxy and galaxy-cluster formation^{3–5}.

Here, we study various other astrophysical consequences of such string scenarios. One of the most serious observational constraints on strings (or conversely, their most promising distinctive observational signature) is the background of gravitational radiation they would produce⁶, which is now detectable because of the precision of the millisecond pulsar. Within a few years, measurements will probably be sensitive enough to exclude any scenario which suggests that strings produce galaxy clustering using only their direct gravitational effects.

We also study some other unusual interrelated consequences of the string picture, including: (1) recoil reaction of gravitational radiation on oscillating loops; (2) damping of the translational energy of such loops by dynamical friction on ordinary matter; (3) accretion of ordinary matter bound to a loop and the motion of a loop within its potential well; and (4) the dissipative coupling of the internal energy of loops to ordinary matter. In some situations, loop oscillations can noticeably affect bound stellar or gaseous systems, although as far as the loops are concerned there are generally no comparable energy-loss mechanisms to the gravitational radiation.

Finally, we consider the possibility of using strings as ‘seeds’ in an alternative theory of galaxy formation, in which they are required only to produce some pregalactic energy sources instead of the whole of the observed clustering energy. This allows the use of much lighter strings and hence avoids, for the time being at least, the gravitational-wave constraint. The minimum value of the string mass expected to have significant astrophysical effects has some interest because string production is predicted in association with a Peccei–Quinn symmetry breaking, generally thought to occur at well below the grand unification scale ($\approx 10^{12}$ GeV to close the Universe with axions)⁷.

General properties of strings

Strings are topologically-stable structures which may appear in the vacuum as a result of domains formed during the breaking of a Yang–Mills symmetry, if the topology of the manifold of degenerate vacua in the broken phase is appropriate. They have a mass per unit length $\mu \equiv \varepsilon/G$, where $\varepsilon \approx (\langle \phi \rangle / m_p)^2$ is the dimensionless amplitude of their gravitational potential, m_p is the Planck mass, and the vacuum expectation value of the Higgs field is $\langle \phi \rangle$. Because of their enormous tension ε/G the network of strings formed in the transition, which may be thought of as a superposition of random walks, strives to reduce its length by ironing out small scale wrinkles at close to the speed of light and allowing strings, when they cross, to intercommute or exchange partners. Strings are forbidden to have ends (at least in the simplest models) so they must either be in closed ‘loops’ or in infinite ‘open’ strings.

A closed loop whose radius matches the current horizon size t_i has a good chance of not intercommuting further with the rest of the network. The behaviour of isolated closed loops is still open to question. They tend to undergo oscillations in which the transverse inertia acts as a ‘weight’ and the restoring force is provided by longitudinal tension. What is not known is how frequently an initial loop configuration chosen ‘at random’ (from the network of self-avoiding random walks) will cross itself and bifurcate into smaller loops, and how frequently it will oscillate indefinitely as a stable object. If the latter case is not very unlikely, the network produces debris of stable oscillating loops smaller than the current horizon which are effectively decoupled from each other and from the rest of the network. These loops eventually decay by gravitational radiation at a time $t_{\text{dec}} \approx \varepsilon^{-1} t_i$. At a time t loops which are larger than $R_{\text{dec}} \approx \varepsilon t$ (the ones currently decaying), and which separated out at $\approx t_i$ have a mean separation (in the long-lived case) of the order of $R_{\text{sep}}(t) = (a(t)/a(t_i)) t_i$, where a is the Friedmann scale factor. During the matter-dominated era (for $t > t_{\text{eq}}$), loops in each logarithmic interval of loop size $R_1 \geq t_{\text{eq}}$ contribute a fraction $\sim \varepsilon$ of the mean cosmological density; for $R_1 < t_{\text{eq}}$, the fraction increases as $R_1^{-1/2}$ (because of the change in the expansion law when these loops entered the horizon), down to R_{dec} , below which it falls off like R .

Although many properties of loops can be estimated only in an order-of-magnitude sense, it is sometimes useful to be reasonably definite in normalization of certain quantities.

(1) We assume that loops form when their radius is equal to H^{-1} (the current Hubble time), and that, on average, one half of such a loop appears in a typical spherical volume of radius cH^{-1} . This leads to an estimate of the mass-density perturbation

by the loops at horizon crossing $(\delta\rho/\rho)_{\text{hc}} = 2\pi\epsilon$.

(2) The value of ϵ required to produce galaxy clustering by gravitational interaction alone depends on the composition of the dark matter, because of the usual damping processes which can take place at high redshift. However, a useful estimate of a lower limit for ϵ can be obtained by assuming a matter-dominated universe of cold particles. Simple scaling derived from the linear-growth model for the perturbations $(\delta\rho/\rho)_{\text{hc}}$ after they enter the horizon gives

$$(\delta\rho/\rho)_{\text{hc}} = (r_0/ct_0)^2 \quad (1)$$

where r_0 is the radius for which randomly sampled spherical volumes have unit variance in density at the present epoch, $(\delta\rho^2)_{r=r_0} = (\rho^2)$. Estimates of the galaxy correlation function show that $r_0 \approx 10 \text{ Mpc } h_{100}^{-1}$ where $h_{100} = H_0/100 \text{ km s}^{-1} \text{ Mpc}^{-1}$ (for a more detailed discussion see ref. 9), so

$$2\pi\epsilon \geq 10^{-5} r_{10}^2 \quad (2)$$

if strings are to produce galaxy clustering by gravity alone. (This estimate applies independently of the probability of loop formation because it only relies on linear growth after t_{hc} .) In what follows we use a fiducial value $\epsilon_{-6} \equiv \epsilon/10^{-6} \geq 1$.

(3) Oscillating loops, in their gravitational interaction with normal matter, behave like a mass $M_l \approx 2\pi\mu R_l$ spread out over the volume covered by the oscillations (in a sense to be described below). It is useful to associate loops with a virial velocity $v_{\text{vir}} \approx \epsilon^{1/2}$, a virial time $t_{\text{vir}} \approx \epsilon^{-1/2} R_l$ and a virial temperature $T_{\text{vir}} \approx \epsilon m_{\text{pr}}$ where m_{pr} is the proton mass. The circular orbital velocity of a particle around a circular loop at the loop radius is

$$v_{\text{vir}} = \sqrt{2GM_l/R_l} \approx 1,000 \epsilon_{-6}^{1/2} \text{ km s}^{-1} \quad (3)$$

(4) Turok⁵ has found exact solutions for which the simple order-of-magnitude estimate for the gravitational-wave decay half-life, τ_{dec} , of loops is remarkably accurate:

$$\tau_{\text{dec}} \approx \epsilon^{-1} R_l \quad (4)$$

to within a factor of two for various modes.

Since loops have a constant $GM/R = \epsilon$, smaller loops have a higher effective local density contrast $M_l/(\rho_m R_l^3) \propto M_l^{-2}$ where ρ_m is the matter density. Loops whose mean density matches the background have a mass $\approx \epsilon^{3/2} M_{\text{H}}$, where $M_{\text{H}} = 4\pi\rho_m t^3/3 = t/2G = 10^{23} M_{\odot} h_{100}^{-1} \Omega$ is the current horizon mass Ω is the cosmic density parameter; loops which are currently decaying have a density contrast $\approx \epsilon^{-1}$, and are very much denser than background matter in their vicinity. Their mass is $M_{\text{dec}} \sim 2\pi\epsilon^2 M_{\text{H}} \approx 6 \times 10^{11} \epsilon_{-6}^2 \Omega h_{100}^{-1} M_{\odot}$, their radius is $R_{\text{dec}} \approx 3h_{100}^{-1} \epsilon_{-6} \text{ kpc}$, and their mean separation is $\approx \epsilon^{1/3} t \approx 30h_{100}^{-1} \epsilon_{-6}^{1/3} \text{ Mpc}$ (loops of this size are the most numerous). If these loops are present in the Universe today, the discussion below indicates that some, at least, should be found in clusters of galaxies, and we expect roughly one loop per rich cluster.

Gravitational wave background

Vilenkin⁶ has sketched out the gravitational wave background expected in the loop scenario. Here we consider several regimes and compare the predictions with current limits from the millisecond pulsar.

Let us suppose that newly-formed loops fall with probability W into the set of infinite-non-self-intersecting trajectories⁸. A fraction W of horizon-sized loops form a long-lived stable population as described above, and all of their rest mass is released eventually as gravitational radiation at z_{dec} . Let the differential energy spectrum of gravitational waves of frequency ω be $\rho_g(\omega)$; define a logarithmic spectrum Ω_g by

$$\rho_g(\omega) d\omega \equiv \Omega_g(\omega) (d\omega/\omega) \rho_c \quad (5)$$

where ρ_c is the critical density. Gravitational waves redshift in exactly the same way as electromagnetic ones, so loops which formed and decayed during the radiation-dominated era pro-

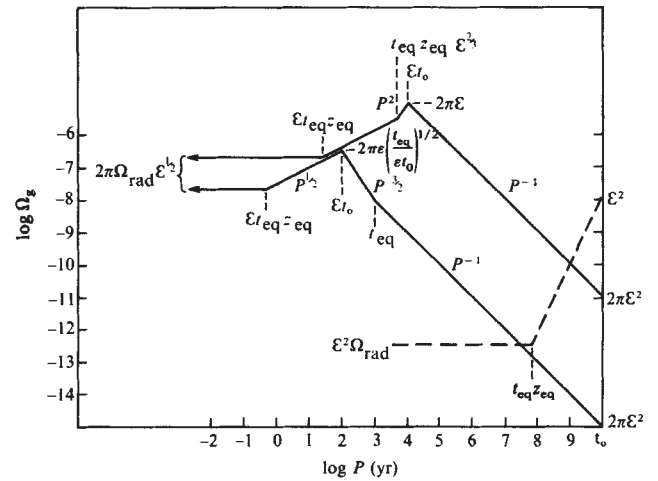


Fig. 1 Spectrum of stochastic gravitational radiation background produced by population of oscillating loops. Logarithmic energy density is plotted against period for two cases, $\epsilon = 10^{-8}$ and 10^{-6} . Inflection points between different power-laws correspond to waves emitted by loops which decayed or entered the horizon at t_{eq} , and loops which are now decaying. The upper curve corresponds to the case of strings important for galaxy clustering, and can be tested against data at $P \sim 1 \text{ yr}$. The dashed line represents the background produced by primordial tensor perturbations in the metric.

duce a background with

$$\Omega_g \approx W \Omega_{\text{rad}} \left(\frac{\delta\rho}{\rho} \right)_{\text{hc}} \left(\frac{z_i}{z_{\text{dec}}} \right) \approx 2\pi W \Omega_{\text{rad}} \epsilon^{1/2} \approx 2 \times 10^{-7} W \epsilon_{-6}^{1/2} h_{100}^{-2} \quad (6)$$

where z_i is the redshift of loop formation, the z factor is the amount $\approx \epsilon^{1/2}$ by which the radiation redshifted between z_i and z_{dec} , and we have used $\Omega_{\text{rad}} \approx 10^{-4.5} h_{100}^{-2}$ and $(\delta\rho/\rho)_{\text{hc}} \approx 2\pi\epsilon$. This flat specimen extends down to waves emitted by loops which formed near the time of the phase transition itself, at temperature $\approx \epsilon^{1/2} m_p$. This corresponds to a present-day period $P_{\text{min}} \approx 10^{-11.5} N^{-1/2} \text{ s}$ (independent of ϵ), where N is the number of particle degrees of freedom. The shape of the spectrum at longer wavelengths depends on whether the loops currently decaying formed before or after t_{eq} (whether $t_{\text{eq}} < \epsilon t_0$ or $t_{\text{eq}} > \epsilon t_0$, the latter case being more likely); these spectra are summarized in Fig. 1, which includes the case considered by Vilenkin. The waves observable with the millisecond pulsar, with periods P of the order of 1 yr, were emitted at a redshift

$$z_{\text{dec}} \approx 2 \times 10^6 \epsilon_{-6} P_y^{-1} N^{-1/6} \quad (7)$$

and originally had a period

$$t_i \approx P_y^2 \epsilon_{-6}^{-1} N^{1/3} \times 10 \text{ s} \quad (8)$$

Therefore, the waves in this scenario are relics of the early stages of the expansion, emitted by loops which formed shortly after nucleosynthesis.

Note, however, that the strings give a gravitational wave background very different from a primordial spectrum of curvature fluctuations (produced at the end of inflation¹⁰, say); in this case, if scalar and tensor modes have comparable metric perturbations $|h_{\mu\nu}|$, the gravitational wave density $\Omega_g \approx h^2(P/t_0)^{-2}$ is of the order of $(\delta\rho/\rho)_{\text{hc}}^2$. Another semi-classical source of perturbations might be the Starobinsky^{11,12} effect, which produces gravitational waves (without scalar fluctuations) which enter the horizon with $\Omega_g \approx (\delta\rho/\rho)_{\text{hc}}^2 \approx (H_{\text{inf}}/m_p)^2$, where H_{inf} is the Hubble constant during inflation. The spectrum from these effects is also plotted in Fig. 1. The short-wave cutoff in this case is $\approx (H_{\text{inf}}/m_p)^{-1/2} P_{\text{min}}$. One can set constraints on H_{inf} using microwave anisotropy measurements (because in this case the waves are already present when the relevant scales enter the horizon), and the millisecond pulsar can only set more severe

constraints if its Ω_g sensitivity goes below $\Omega_{\text{rad}}(\delta T/T)^2$ where $\delta T/T$ is the sensitivity of anisotropy measurements. In these scenarios, the microwave background isotropy is likely to remain a more sensitive constraint.

If W is sufficiently less than 1 there will also be a contribution from recently-formed loops. Let us consider a scenario in which an oscillating loop bifurcates frequently, leading to a 'cascade' of loop divisions to successively smaller scales. A loop of mass M will on average bifurcate $\approx \log_2 W$ times until there are $\sim W^{-1}$ daughters and there is a good chance that one of its progeny, of total energy $\approx MW$, is oscillating in a mode where it is stable. Each successive division of the remaining progeny will leave behind some stable loops, which contain a total mass $\approx MW$ in each logarithmic 'generation'. In general, each division converts some fraction $1-f$ of the loop length into translational motion of the succeeding generation, so that after n divisions loops are moving with relativistic γ -factors $\sim f^{-n}$. The stable loops radiate their rest mass into gravitational waves, as usual, but their radiation, even if emitted isotropically in their rest frame, is now beamed into a solid angle γ^{-2} and blueshifted (within the beam) in frequency by a factor γ . The resulting background has a spectrum

$$\Omega_g \approx \min [\varepsilon^2 W(P/t_0)^{1/\log_2(f^2/2)}, \varepsilon W]$$

which at sufficiently high frequencies could, in principle, be larger than the 'primordial' contribution of equation (6). However, in general, this background is not stochastic; only a small fraction of observers lie within the beam of a radiating loop. In principle, Earth-based gravitational wave antennas might pick up occasional events from loop events of this type, coming from redshifts $\leq 10^6$, if W is moderately small. There is, however, only a small range of W in which these events could occur where one would not encounter a more serious constraint from the γ -ray background, as we now explain.

After $n \sim W^{-1}$ bifurcations, the entire rest mass of the original loop is locked up in non-self-intersecting loops. For $W \ll -\log(f/2)/\log(\langle\varphi\rangle t_0)$, most of the rest mass of the original loop remains when the daughter loops become so small that non-gravitational interactions become important at $R_1 = \langle\varphi\rangle^{-1}$, at which point the energy is presumably radiated in a complicated mixture of coherent vacuum phase waves and high-energy particles. Thus for very small W , the best constraint is likely to come from the γ -ray background produced by the smallest loops in the cascade. Particles with energy of the order of $\langle\varphi\rangle$ would be degraded in $\ll t_0$ down to $\sim 10^5$ GeV by pair production on the non-thermal radio-wave background and microwave background; below this energy (corresponding to pairs produced just over the peak of the blackbody) the mean free path for this process exceeds a Hubble length, and pairs lose most of their energy by inverse Compton scattering on the microwave photons. The resulting γ -ray spectrum peaks at 10^5 GeV with $\Omega_\gamma \approx \varepsilon$ and falls off like $\Omega_\gamma \propto E_{\text{pair}} \propto E_\gamma^{1/2}$ at smaller energies. Observations of cosmic rays near the peak energy constrain ε to be $\leq \Omega_\gamma \leq 10^{-7}$.

Thus very small values of W are already ruled out if the string scenario is to work at all. Recent simulations also indicate that W is probably not smaller than about 10% (A. Albrecht and N. Turok, in preparation), so the gravitational wave background given by equation (6) is a fairly firm prediction of the string scenario. Apart from the uncertainty associated with W , the other major numerical uncertainty in estimating their backgrounds lies in the probability of loop formation. We have assumed above that $1/2$ loop of radius ct forms, per spherical volume of radius ct , when the expansion rate is $H = t^{-1}$. The actual number could be smaller than this, but not by very much; this is another question which can be answered using numerical simulations.

Detection of gravitational waves

We base our discussion on detecting gravitational waves using the millisecond pulsar from earlier work¹³⁻¹⁵. Suppose we observe a clean pulsar for a time T , and, after allowing for

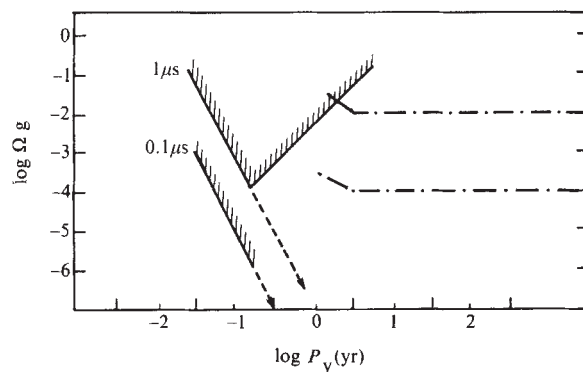


Fig. 2 Current and projected sensitivity of millisecond pulsar timing to gravitational wave background at different wavelengths. Curves are plotted for $\alpha = 2\pi$, $T_y = 1$, and $R = 1$ and $0.1 \mu\text{s}$; dotted lines show expected improvement as data accumulation time T increases, for constant R . Improvement at shorter periods may also be expected by distinguishing various forms of noise in the timing residuals. Limits at larger periods arise from cubic residuals induced by long-wavelength waves. Also shown is the projected optimal sensitivity (dot-dash lines) of the binary pulsar if its orbital clock can be calibrated (the two estimates correspond to different signs for the clock residuals, which are limited by different effects^{14,15}.)

known intrinsic period variations of the pulsar ($\dot{P}$ and $\ddot{P}$) and for standard ephemeris corrections, we find a mean-square timing residual $\langle R^2 \rangle$ (that is, a mean square phase residual $\langle R^2 \rangle (P/2\pi)^{-2}$, where P is the pulsar period). Such a residual can be produced by a stochastic background of gravitational waves; in fact the residual produced by waves with angular frequencies higher than α/T is¹⁴:

$$\langle R^2 \rangle = \frac{32\pi}{3} \rho_c \int_{\alpha/T}^{\infty} d\omega \frac{\Omega_g(\omega)}{\omega^5} \left(1 - \frac{\sin \omega T}{\omega T} \right) \quad (9)$$

assuming isotropy and averaging over random phases and polarizations.

Using $H^2 = 8\pi G\rho_c/3$, and assuming $\Omega_g = \text{const}$ and $\alpha > 1$, this becomes

$$\Omega_g = \langle R^2 \rangle T^{-4} \alpha^4 H^{-2} \quad (10)$$

(where all of the numerical coefficients have cancelled), or in units appropriate to the millisecond pulsar 1937+21 (refs 16, 17, and J. H. Taylor, personal communication)

$$\Omega_g = 10^{-7} h_{100}^{-2} \alpha^4 T_y^{-4} R_{\mu\text{s}}^2 \quad (11)$$

where T is measured in yr, and R in μs . Currently, pulse arrivals are only measured to an accuracy of $R_{\mu\text{s}} \sim 1$ because of time variations in the pulse spectrum induced by interstellar scintillation, which lead to imperfect removal of the effects of interstellar dispersion. However, instrumental improvements will probably reduce $R_{\mu\text{s}}$ to ≤ 0.1 .

Even the millisecond pulsar is not 'clean' in the above sense, because of residuals introduced by secular effects whose intrinsic parameters are unknown, such as the $\dot{P}$ of the pulsar, which must be fit to the data. One really needs to know the value of Ω_g which would be likely (at a given confidence level) to have produced residuals in excess of those which remain after one has subtracted a best-fit quadratic from the raw residuals. Thus, in using real pulsar data to set an upper limit on Ω_g , the 'safe' choice of α , and that of ref. 14, is $\alpha = 2\pi$ (wave period at cutoff = period of observation), because waves of this high a frequency could not accurately mimic a simple quadratic fit. This is perhaps the first instance in which a precise statistical analysis²³ of these limits is worthwhile, as the constraint on string models is such a sensitive function of α . Meanwhile, Fig. 2 presents an estimate of the current and projected sensitivity of the pulsar observations.

The prospects for achieving levels where string models will be seriously constrained are apparent from the above formulae,

which yield

$$\varepsilon < 2.5 \times 10^{-7} (T_y/\alpha)^{-8} R_{\mu s}^4 \quad (12)$$

In this formula, R is the residual after fitting for known secular effects, and may begin to increase with time due to some source of noise other than gravitational waves. However, if R does not increase too rapidly, even safe choices of α only require longer integrations (of the order of α yr) to reach the level where values of ε required to produce galaxy clustering gravitationally could be ruled out with a high level of confidence.

The predicted spectrum of gravitational radiation leads to residuals increasing quite rapidly with time—at least as fast as $R \propto t^2$, somewhat faster for $\varepsilon \leq 10^{-6}$. Variations in interstellar electron density lead to residuals which increase only as $R \propto t^{0.8}$, and in any event have a spectral signature which aids in their removal¹⁸. Imperfections in the terrestrial clock standards and Solar System ephemeris are an important source of error; they are currently at the level of $\sim 0.3 \mu s$ over 1 yr (D. C. Backer, personal communication). A confident detection of gravitational waves probably requires correlating residuals with other equally clean pulsars so the possibility of intrinsic pulsar variations, and the effects just mentioned, can be removed.

It is also possible that the generation of Earth-based gravitational-wave detectors (~ 5 -km laser interferometers) could detect a stochastic background with $\Omega_g \approx 10^{-7}$ at $P \sim 10^{-2}$ – 10^{-3} s. With $\Omega_g = h^2 (PH_0)^{-2}$, this requires a broad-band sensitivity to metric deformations $h = 10^{-23}$ – 10^{-24} .

Dynamical friction

Loops are born with initial centre of mass velocities v_i close to the speed of light, because of the acceleration given by the last intercommute which formed them and subsequently slow time $v \propto a^{-1}$ due to the expansion. Loops also suffer dynamical friction on cold collisionless matter^{19,20}. The dynamical friction rate is about

$$t_{df}^{-1} \approx 2\pi^{3/2} G^2 \rho M \ln \Lambda / v^3 \quad (13)$$

where v is the peculiar velocity of the loop and $\Lambda \equiv v/R_i$ is the range of relevant impact parameters. If $t_{df} < H^{-1}$, the peculiar velocity of a loop decays rapidly to the velocity dispersion of the background material. This occurs if

$$(H t_{df})^{-1} \approx v_i^{-3} R_i H (a(t)/a(t_i))^3 \varepsilon \ln \Lambda \quad (14)$$

exceeds unity. For a matter-dominated universe of cold matter, loops with initial γ factors of the order of unity stop just before they decay at $t \approx \varepsilon^{-1} R_i$.

(There is also an additional direct dynamical friction on strings moving through matter due to the 'wake' of material deflected behind it²⁰. This dissipation is important only for one or two oscillations after a loop enters the horizon, although it may produce observable discontinuities in the microwave background temperature²¹.)

However, the dynamical friction is much less effective during the early radiation-dominated phase. If the background medium is treated as collisionless particles with speed $\tilde{v}$, then the dynamical friction slowdown time scale for a loop moving with speed $v < \tilde{v}$ is independent of v (and proportional to $\rho^{-1} \propto t^2$). For a given ρ this time scale is longer by $(\tilde{v}/v)^3$ than it would be if the background particles were cold. Dynamical friction is reduced further for subsonic motion of a shallow potential well through a collisional gas²² (such as the baryon+photon fluid before recombination). Thus, in the early universe it is the neutrinos, and not the photon-plus-baryon fluid, which produce the main drag. Dynamical friction is never efficient before recombination except in a model containing cold dark matter.

The anisotropy in the gravitational wave energy (and momentum) radiated in a single oscillation may be of the order of unity and, therefore, a recoil velocity of $\Delta v_s \approx \varepsilon$ is induced by each oscillation⁵. The cumulative effect depends on whether there is long-term stability in the mode of oscillation and its orientation. If the oscillations are strictly periodic (at least for a time comparable with the gravitational wave decay time scale), then the

radiation is repeatedly beamed in exactly the same direction producing an acceleration $\approx c/t_{dec}$. Thus a loop initially slows down like $v \propto a^{-1}$, reaches a minimum velocity $v_{min} \approx \varepsilon^{2/5} v_i^{3/5}$, and shoots up to a velocity $\sim c$. In this case, loops with $v_i \approx c$ never slow down sufficiently for dynamical friction to have any effect. In the opposite extreme, when successive oscillations are completely uncorrelated, the velocity random walks, and can build up only to $\sim \varepsilon^{1/2} c$ on the overall gravitational wave time scale. Real loops lie between these extremes.

Consider now what happens if we include both dynamical friction and recoil forces on a loop. The recoil velocity attainable in a time $t_{dr} (< t_{dec})$ is of the order

$$v_c = c(t_{dr}/t_{dec}) \min [1, (t_{coh}/t_{dr})^{1/2}] \quad (15)$$

where t_{coh} is the effective coherence time for the oscillations. If this velocity exceeds $\tilde{v}$, then the loop can run away in the sense that dynamical friction will never again be important. In the opposite case, where $\tilde{v} > v_c$, recoil will accelerate a loop to the point where the dynamical friction just damps the energy being added by recoil, rather like brownian motion in a viscous fluid. The actual value of $\tilde{v}$ is unknown, but in the case of cold dark matter such as axions could be arbitrarily small.

A loop whose gravitational radiation recoil is $\ll c/t_{dec}$, or whose initial velocity is sufficiently small, may 'accrete' a halo of matter around it. A loop can accrete its own mass when it slows to the Hubble velocity across a mass of matter equal to the loop mass. For $v_i \leq \varepsilon_c^{1/6}$ this occurs by redshifting before coherent recoil begins to take effect, but for $v_i \approx c$ it occurs at $\approx t_{dec}$, and even then only if $v_c < \tilde{v}$. The depth of the potential, and the total mass accumulated, depend on the details of when a loop actually slows.

Would the recoil from gravitational radiation eject a string loop from a virialized system? Even if recoil acts in a constant direction, this requires the recoil to produce an acceleration which is sufficient for it to break out of the potential well—that is $\varepsilon/l > v_{vir}^2/l = (M_{mat}/M_l)\varepsilon/l$. Thus a string cannot (unless it breaks into two) escape from a potential well which it does not dominate, although the recoil may cause it to reside away from the centre of the potential well. The effect of the beaming would be to cause the entire virialized system to recoil.

Dissipative heating by oscillating string

After accreting a halo of matter around itself, how does a loop interact with the ordinary matter bound to it? Can gravitational coupling of a loop to ordinary matter enable it to dissipate its rest mass more quickly than an isolated loop?

Goddard *et al.*²⁴ discovered that, surprisingly, strings of constant invariant length—for our purposes, this means perfectly nondissipative loops, with no gravitational interactions—obey a perfect nondissipative linear wave equation, even for large-amplitude waves. The physical reason for this behaviour is that, locally, the inertia and the tension of the string must always be exactly orthogonal because of restrictions imposed by Lorentz covariance. As a consequence, Kibble and Turok⁸ showed that the motion of such a perfect loop is described by advanced and retarded waves travelling with arbitrary waveforms (except that some will lead to self-intersection) in the two directions around the loop. Because the solutions are exactly periodic, the loop traces out repeatedly the same contorted surface, and in fact has a time-averaged gravitational field equivalent to a surface whose local mass density acts like the square of the local transverse velocity²⁵. Perfect coherence is maintained indefinitely, so the orientation of this surface is stationary for a perfect loop in a particular Lorentz frame of reference.

We have already seen that the long-term coherence of the direction of gravitational beaming makes a crucial difference in their behaviour (whether they rocket off or whether they stop and accrete matter). Now we find that this coherence is also of crucial importance for the dissipative interaction of loops with matter, because whereas a perfect loop has vanishing power at frequencies $\ll R_l^{-1}$ even a slight perturbation can generate components which vary over a longer time scale, comparable to the

orbital time scales of stars. This allows for much more efficient coupling.

A string undergoing exactly periodic oscillations will, in effect, produce a quadrupole (newtonian) component of the gravitational potential which oscillates on a time scale R_1 . This time scale is very short compared with the dynamical time scale (in most applications), so the effect is merely to produce small amplitude quasi-periodic shear distortions. Let the mass of (non-string) material within a sphere of diameter R_1 be $x \times$ (mass of string loop). Then if $x < 1$, the gravitational binding is due to the string; otherwise the string is just a perturbation:

$$v_{\text{vir}} \approx \varepsilon^{1/2} \times \max[1, x^{1/2}]c \quad (16)$$

The velocity perturbation induced by the string in a time R_1 is $\Delta v = \varepsilon$ (this is true for all values of x , though not completely obviously so). The corresponding shear amplitude is $\sigma \approx \varepsilon$.

The amount of dissipation depends on the ratio $y = t_{\text{coll}}/R_1$ where t_{coll} is the particle collision time. If this is $\gg 1$ (as, for instance, in stellar-dynamical systems) then the dissipation is small; also, if it is $\ll 1$, so that the pressure remains isotropic, there is again no dissipation. The dissipation is maximized if $y \approx 1$, but even then the internal energy of the cloud is increased by only $\Delta\sigma/\sigma \approx \sigma^2$ per oscillation. There are two contexts when we would indeed expect $y \approx 1$: hot gas in clusters of galaxies, when the ions and electrons have mean free times against Coulomb scattering of order l/c ; and pre-combination baryon clouds of $\approx 10^6 M_\odot$ where the photons provide the main internal pressure, and have mean free paths of order the scale of the system. The motion of the loop also leaves a 'wake'²⁰, or shear-free velocity perturbation $\Delta v \approx \varepsilon$ normal to the swath swept out by the string. The energy dissipated from this effect would also be of order ε^2 per oscillation, for $y \gg 1$ and $x \ll 1$.

As the strings decay through gravitational radiation after $\sim \varepsilon^{-1}$ oscillations, this suggests that fractional change in the energy content of a virialized system due to the oscillations of a perfect loop is negligible (a fraction $\leq \varepsilon^{1/2}$ of the binding energy of the matter).

Gravitational interactions of the loop's mean newtonian quadrupole field are likely to have much stronger effects, but their character depends strongly on the long-term coherence over many oscillations. For strings of the type discussed by Kibble and Turok, the orientation and amplitude of the time-averaged newtonian quadrupole moment probably remains coherent over a time $\sim t_{\text{dec}}$ if gravitational radiation losses dominate. The surface swept out by the string thus forms a rigidly fixed gravitating shell. If matter in its vicinity has a rotating ellipsoidal figure, the loop will torque the matter through dynamical friction (even if $x > 1$) until it is rotating by an angle $\approx \varepsilon^{1/2}$ per orbital time.

Other types of string not obeying strict local covariance are likely to be less coherent than these loops. The optimum time scale t_{coh} for the coherent variation of the newtonian quadrupole to interact most strongly with matter is, of course, $t_{\text{coh}} \approx t_{\text{vir}}$; in this case the string could, in principle, disperse the matter within one orbital time, if $x \ll 1$. However, it is possible that the coherence time is very short compared with t_{vir} . The extreme case—and the case of weakest coupling for short t_{coh} —is to set $t_{\text{coh}} \sim R_1$. The main effect is then contributed by variations in the loop's quadrupole moment averaged over time $T \approx t_{\text{vir}}$; the matter now experiences a randomly oriented shear distortion $\sigma \approx \varepsilon^{1/4}/\max(1, x^{3/4})$ every orbital time. Moreover, the distortion is dissipative even if $t_{\text{coll}} \gg t_{\text{vir}}$ because of violent relaxation. We then have a fractional increase in the matter's energy of order $\sigma^2 \approx \varepsilon^{1/2}/\max(1, x^{3/2})$ every orbital time, which for $x \ll 1$ amounts to its entire binding energy during the lifetime of the loop. The interaction of a loop with the central galaxy in a cluster, for example, would be sufficient to evaporate a substantial fraction of its matter in a Hubble time into an extended envelope. The loop could also provide a significant heat source for the cluster gas, although maximal dissipation would now occur for $t_{\text{coll}} \approx t_{\text{vir}}$.

Minimum value of ε

It is possible that loops would establish 'seeds' of some kind (meaning astrophysical sources of energy) even if ε were too small to have a direct influence on galaxy clustering. The energy sources could then be used to generate clustering by some secondary process.

A minimum value of ε for which anything would happen even after decoupling is that for which $T_{\text{vir}} \approx T_{\text{rad}}$, because matter cannot cool radiatively below T_{rad} and if it can be pressure supported at that temperature against a loop's gravity no collapse occurs. Thus at $z = 30$ (say) $T_{\text{rad}} \approx 100$ K so baryon clouds with $\delta\rho/\rho > 1$ form around loops even if $\varepsilon \approx 10^{-12}$ and the vacuum expectation value is as small as $\langle\varphi\rangle \approx 10^{-6} m_p \approx 10^{13}$ GeV; it is sufficient to have $v_{\text{vir}} \approx 1$ km s⁻¹. However, this condition is not sufficient for further collapse and fragmentation. The matter heats up to T_{vir} and again nothing happens unless there is a cooling mechanism at that temperature which can permit the gas to radiate its binding energy in a free-fall time. Various authors have considered rates for cooling in primordial gas, in which cooling is dominated by molecular hydrogen, whose abundance is determined by a charge-exchange reaction catalysed by free electrons. We use the formula given by Blumenthal *et al.*²⁶, $t_{\text{cool}} < t_{\text{dyn}}$ if

$$M > M_{\text{cool}} = 4.1 \times 10^7 M_\odot \left(\frac{n_e/n_H}{10^{-4}} \right)^{-0.625} (\Omega h^2)^{-0.917} \\ \times \left(\frac{\Omega_b/\Omega}{0.1} \right)^{-1.04} \left(\frac{1+z}{10} \right)^{-2.75} \quad (17)$$

Strings characteristically generate density fluctuations leading to systems which collapse when $M \approx \varepsilon^{3/2} M_H(z)$. Now $M_H \propto z^{-3/2}$ so it is clear that a lower ε is permitted by the cooling argument for higher z . The lowest interesting value of ε will be that which has $M_{\text{cool}} \approx \varepsilon^{3/2} M_H(z)$ at the epoch when $T_{\text{vir}} = T_{\text{rad}}$. This works out to be a redshift $z \approx 100$ and $\varepsilon \approx 10^{-11}$, which may be low enough to be attributable to Peccei–Quinn symmetry breaking. The gravitational wave background for this low an ε is probably (though not necessarily) inaccessible to the millisecond pulsar.

Conclusions

If pulsar limits on Ω_g go below $\sim 10^{-7}$, as it seems will happen soon, then cosmic strings will be pretty well ruled out as candidate sources for any large-scale fluctuations having a significant direct impact on galaxy clustering. At present, however, there is still the possibility that their gravitational waves will start to appear in timing residuals during the next few years. This motivation provides a strong incentive to find at least one other very clean pulsar (preferably several) so that a more definitive signature may be established.

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Sequential late Cenozoic structural disruption of the northern Himalayan foredeep

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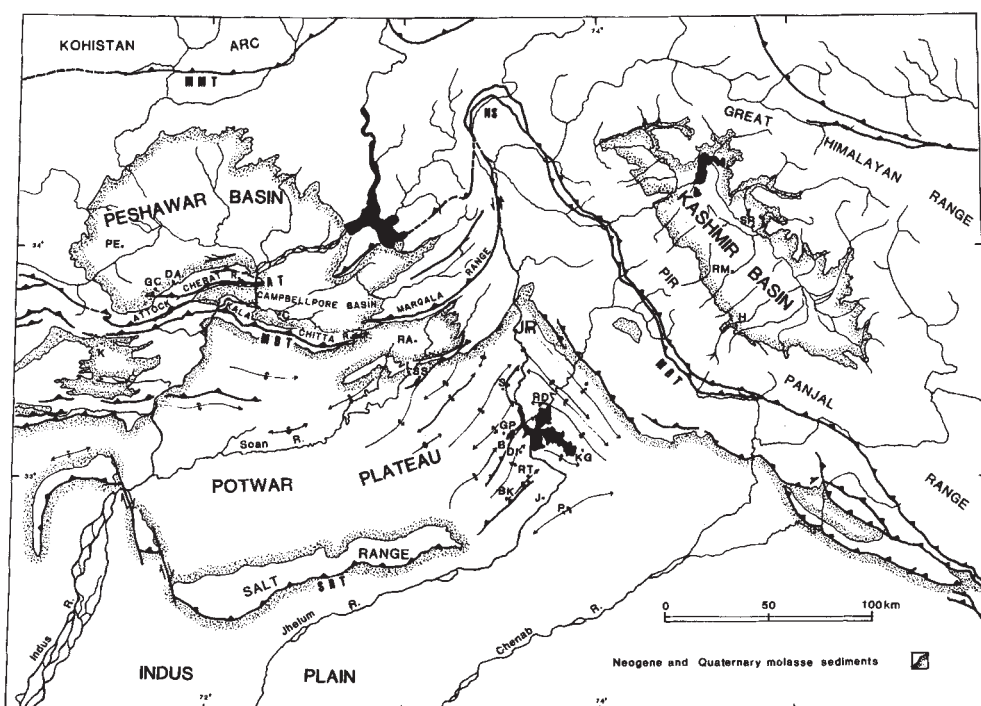
Chronologies for the Siwalik molasse and intermontane basins along the southern margin of the Himalaya and Hindu Kush Ranges constrain the timing and pattern of facies migration and structural disruption of the Indo-Gangetic foredeep. This synthesis indicates that quiescent intervals are punctuated by pulses of rapid deformation as thrusting migrates in a stepwise fashion across the foredeep.

THE large-scale southward migration of tectonic deformation away from the axis of the Himalayan orogen has long been recognized^{1–4}. Extensive thrusting and nappe development in the vicinity of the Main Central Thrust in the Himalayas and of the Main Mantle Thrust in the Hindu Kush occurred primarily during the middle Cenozoic^{4–10}. In contrast, the development of the Main Boundary Thrust (MBT) and related thrusts along the northwestern margin of the Indo-Gangetic foredeep (Fig. 1) has been largely confined to the Plio-Pleistocene^{1,2,4,10}. Using magnetic-polarity stratigraphy and fission-track dating, Johnson *et al.*¹¹ and Opdyke *et al.*¹² have shown that close temporal constraints can be placed on tectonic events recorded by the molasse sediments of the western Himalaya. However, a precise

chronology for the succession of events by which thrust faulting disrupted sedimentation within the molasse basin has not been previously delineated. The synthesis presented here is based on the analysis of lithofacies, provenance, and accumulation rates of strata in 14 recently dated sections. Integration of these data reveals the detailed timing of the progressive migration of deformation across the proximal foredeep margin in northern Pakistan and northwestern India during the past 5 Myr (Fig. 1).

Two distinct depositional settings, the largely undisturbed foredeep and the intermontane basins, are found adjacent to the deformational front. In the Indo-Gangetic foredeep, molasse sediments are typically characterized by sheet sandstones representing low-sinuosity, laterally-migrating rivers^{13,14}, siltstones

Fig. 1 Map of the northwestern portion of the Indo-Gangetic foredeep, the southern margin of the Himalaya and Hindu Kush, and the major intermontane basins in the vicinity of the North-west Syntaxis (NS). The major anticlinal axes in the deformed molasse sediments, as well as major thrust faults (barbed lines) and strike-slip faults in the region surrounding the Jhelum Re-entrant (JR), are delineated. During the Plio-Pleistocene, structural deformation has progressively migrated southwards across the foredeep in response to the ongoing Indo-Asian collision. Major thrust faults: AT, Attock Thrust; MBT, Main Boundary Thrust; MMT, Main Mantle Thrust; SRT, Salt Range Thrust. Locations of measured and dated stratigraphical sections: B, Bangala; C, Campbell-pore; DA, Dag; DI, Dina; GP, Ganda Paik; GC, Garhi Chandan; H, Hirpur; KG, Kas Guma; L, Lei; RD, Rata-Dadial; RT, Rohtas; RM, Romushi; S, Sakrana; SS, Soan Syncline. Other localities: BK, Basawa Kas; J, Jhelum; P, Pabbi; PE, Peshawar; RA, Rawalpindi; SR, Srinagar.



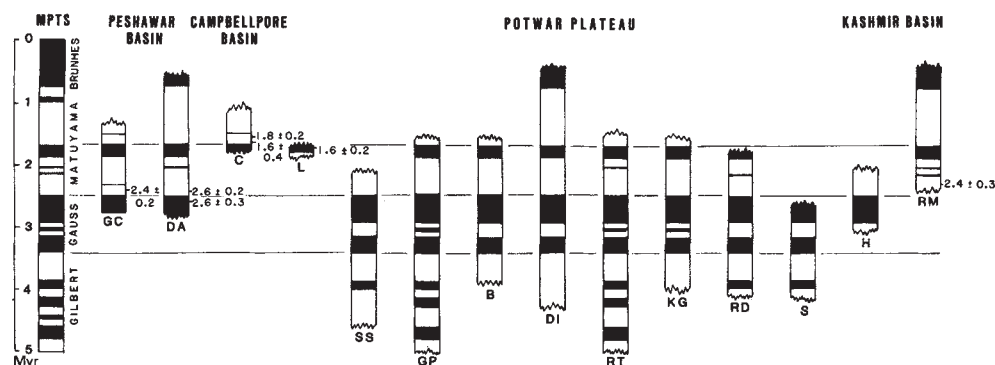


Fig. 2 Comparisons of locally determined magnetic-polarity stratigraphies (MPSs) with the magnetic-polarity time scale^{41,42}. Fission-track dates on volcanic ashes are shown with 2σ errors at their appropriate stratigraphic positions. The magnetostratigraphical correlations are based on recognition of identifiable chrons or subchrons and are aided by both fossil occurrences and the frequent presence of prominent ashes straddling the Gauss–Matuyama transition. The fission-track dates from Lei and from the lower ash at Campbellpore are reported in ref. 29. The Soan Syncline section is from ref. 31. For a detailed discussion of other sections and fission-track dates, see refs 13 and 24. The local MPSs are arranged as they would be encountered along a traverse from the Peshawar Basin southeastwards to the Potwar Plateau and then northeastwards to the Kashmir Basin. In order: MPTS, magnetic-polarity time scale; GC, Garhi Chandan; DA, Dag; C, Campbellpore; L, Lei conglomerate; SS, Soan Syncline; GP, Ganda Paik; B, Bangala; DI, Dina; RT, Rohtas; KG, Kas Guma; RD, Rata-Dadial; S, Sakrana; H, Hirpur; RM, Romushi. Note that based on the sediment accumulation rates in the dated portion of the Hirpur sequence, the base of the underlying conglomeratic and mudstone section is interpreted to be ~ 4 Myr old¹⁹.

with numerous palaeosol horizons developed on broad, interfluvial floodplains^{15–17}, and subsidiary ribbon sandstones representing higher sinuosity rivers¹⁷. Lacustrine strata are virtually absent within the molasse sediments^{11–17}. The molasse sequence, 6,000 m thick, is exposed in the Himalayan foothills, where it is termed the Siwalik and Rawalpindi Groups¹⁸. In contrast, the intermontane basins, such as the Kashmir and Peshawar Basins, are dominated by proximal alluvial-fan facies, intermediate braided-river sediments, and extensive high-sinuosity fluvial and shallow lacustrine sediments that extend across the central portion of the basins^{19–21}. These sequences occur in tectonically ponded valleys that lie inside the deformational front of the Himalayan fold-and-thrust belt. It is the predominant control on the style and rate of sediment accumulation exerted by the tectonic environment that permits us to unravel the patterns of regional tectonic evolution by dating strategically located stratigraphical sections.

Western limb of Jhelum Re-entrant

The Potwar Plateau represents the outermost and youngest of the series of intermontane basins in the western Himalaya. It is separated from the southernmost ranges of the Hindu Kush by

the Peshwar and Campbellpore intermontane basins (Fig. 1). Earthquake-hypocentre distributions suggest that the Peshawar Basin is underlain by a subhorizontal detachment surface^{22,23}. This surface is inferred to continue southward as a largely aseismic detachment beneath the Potwar Plateau and to break the surface at the southern edge of the Salt Range. The Attock Thrust and the MBT (Fig. 1) are inferred to represent splays off this detachment surface^{22,23}. Late Cenozoic movement along these thrusts has uplifted the Attock–Cherat Range and the Kala Chitta–Margala Range which define the southern margins of the Peshawar and Campbellpore Basins, respectively (Fig. 1). To constrain the deformational chronology of the northern foredeep, we have dated sections: (1) within the Peshawar and Campbellpore Basins; (2) near the bounding faults; and (3) across the Potwar Plateau.

Along the northern flank of the Attock Range in the Peshawar Basin (Fig. 1), mid-to-late Pleistocene uplift has exposed intermittently the unconformable contact of the basin-filling sediments with the underlying Oligocene–Miocene bedrock^{24,25,43}. Two magnetostratigraphical sections from this area commence in the upper Gauss chron, as indicated by three fission-track dates on ashes straddling the overlying reversal interpreted as

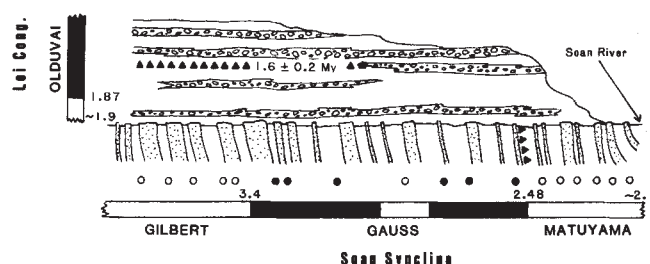


Fig. 3 The northern limb of the Soan Syncline, where nearly vertical strata of typical Siwalik molasse sediments are truncated and overlain by the generally underformed Lei conglomerate. Only the upper portion of $>3,000$ m of molasse sediments is shown. ▲, Enclosed volcanic ashes. The magnetostratigraphy³¹ indicates that these strata encompass the Gauss chron and that the youngest preserved sediments extend into the lower Matuyama chron, probably to ~ 2.1 Myr. The overlying Lei conglomerate includes an ash dated at 1.6 ± 0.2 Myr (ref. 29) on the basis of which the normal-polarity magnetozone is interpreted as the Olduvai subchron. The base of the Lei conglomerate must predate the Olduvai and is interpreted as ~ 1.9 Myr. Thus, between 2.1 and 1.9 Myr ago, $>3,000$ m of uplift and erosion (mean minimum rate of 15 mm yr^{-1}) occurred as the Soan Syncline was strongly compressed in response to thrusting along the MBT.

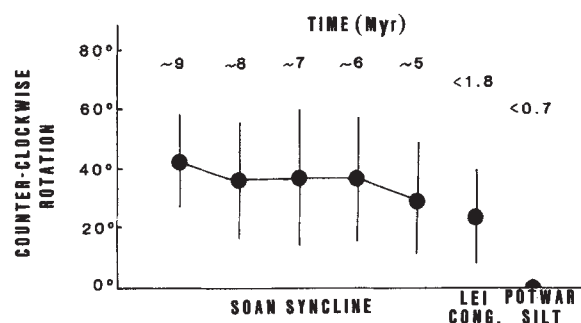
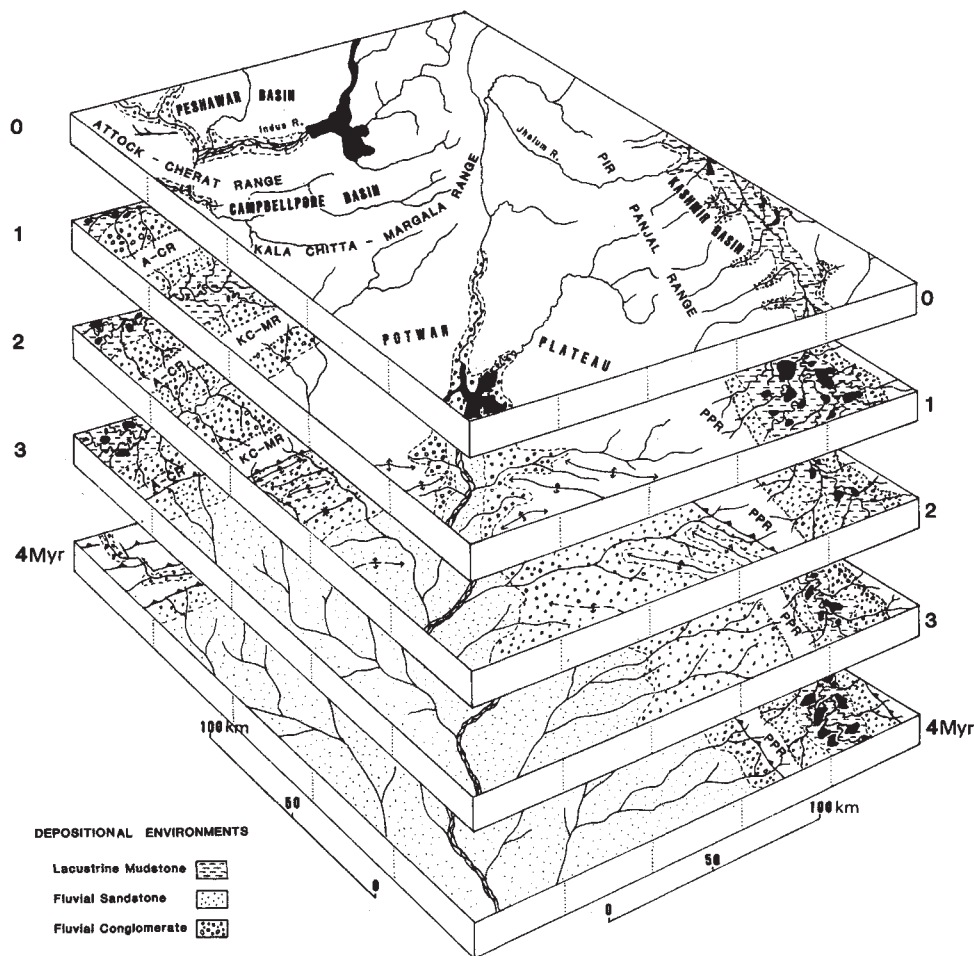


Fig. 4 Post-depositional rotation of foredeep sediments in the vicinity of the Soan Syncline. Amount of rotation is based on the deviation of the mean orientation of the normal and reversed magnetic sites from a given time interval with respect to the theoretical north–south axial dipole. The chronologically constrained data indicate that up to 15° of counterclockwise rotation occurred between 9 and 2 Myr ago. However, the most rapid rotation of $>20^\circ$ occurred between 1.8 Myr and the present, following the development of the Soan Syncline.

Fig. 5 Reconstructed structural and depositional relationships in the vicinity of the North-west Syntaxis at 1-Myr intervals during the past 4 Myr. Changes in facies relationships and the pattern of structural deformation are depicted adjacent to thrusts active at each interval. The zone of molasse deposition beyond the intermontane basins becomes increasingly restricted as thrusting and anticlinal growth migrate across the foredeep. Conglomerate facies and strong structural deformation in positions proximal to active faults grade laterally to indisturbed sheet sandstones in distal settings. A-CR, Attock–Cherat Range; KC-MR, Kala Chitta–Margala Range; PPR, Pir Panjal Range.



the Gauss–Matuyama transition (Fig. 2). Based on the estimated age of the basal depositional contact at Garhi Chandan, intermontane sedimentation in the Peshawar Basin is inferred to have begun ~3.0 Myr ago. Before this, movement along the Attock Thrust (Fig. 1) had caused uplift of the ancestral Attock Range and generated a tectonic depression on the northern side of the uplifted terrane. This tectonic event ponded the fluvial systems which had previously drained across the region and localized sediment accumulation in the newly formed basin.

The Campbellpore Basin is an elongate depression enclosed by the Attock Range to the north and the Kala Chitta–Margala Range to the south (Fig. 1). Despite the brevity of the sections along the western Haro River^{24,26–28} (Fig. 2), the presence of several volcanic ashes permits precise dating of the basin fill^{24,29} and indicates that sedimentation in the Campbellpore Basin had commenced by ~1.8–1.7 Myr ago. We can conclude that the thrusting along the MBT (Fig. 1) which uplifted the Kala Chitta Range and delimited the southern margin of the Campbellpore Basin had commenced at least 1.9 Myr ago.

Further information on the onset and duration of thrusting is provided by a section on the southern side of the MBT. The Soan Syncline (Fig. 1) is located ~20 km south of the MBT near Rawalpindi, where it is developed in sediments of the Upper Siwalik Group. The Soan Syncline is strongly asymmetric with a vertically standing northern limb and a gently dipping southern limb³⁰. The magnetostratigraphy of the Upper Siwalik sediments in the northern synclinal limb³¹ indicates that the youngest deformed strata are ~2.1 Myr old (Figs 2 and 3) and that the sequence, which is >3,000 m thick, extends well into the Upper Miocene¹³. The horizontally-bedded Lei conglomerate unconformably overlies the vertical strata of the northern limb of the syncline. A fission-track date of 1.6 ± 0.2 Myr on an enclosed ash²⁹ indicates that the normal-polarity interval is the Olduvai subchron and that the oldest strata of the Lei conglomerate are likely to be ~1.9 Myr old (Figs 2, 3).

These chronological data delineate a very brief interval of rapid tectonic deformation in the latest Pliocene. During a period of ~200,000 yr beginning around 2.1 Myr ago, >3,000 m of structural relief was developed and was accompanied by rapid erosion. By 1.9 Myr ago, the deformed sequence had been truncated by erosion, and the Lei conglomerate spread southward across the area (Fig. 3).

We interpret the deformation of the Soan Syncline as a response to the initiation of major movement along the MBT around 2.1 Myr ago. The Lei conglomerate represents the proximal, transgressive facies shed from the rising Kala Chitta–Margala Range to the north. The horizontal aspect of the Lei conglomerate today, the gentle dips (<5°) found in the intermontane sediments of the Campbellpore Basin^{22,25,26}, and the absence of conglomerates younger than 1.6 Myr (Fig. 2) suggest that major movement along the MBT was short lived and had largely ceased by the end of the Pliocene. Following deposition of the Lei conglomerate, major rotation of the Soan area began (Fig. 4). We view this rotation as coincident with the propagation of the detachment surface^{22,23} beneath the Potwar Plateau towards the modern Salt Range and with the resulting development of the Potwar Plateau as an allochthonous unit.

During the Pleistocene, tectonic disruption migrated systematically southwards across the central and eastern Potwar Plateau. Three magnetostratigraphical sections^{13,32} from the eastern Potwar (Fig. 2) constrain this history of tectonism. Two intervals of conglomeratic sedimentation are present in most sections: one in the upper portion of the conformable molasse sequence (sometimes intraformational in nature) and one overlying the deformed and eroded molasse strata. The first appearances of conglomerates are time-transgressive events related to either an influx from extrabasinal, bedrock uplifts or intrabasinal deformation. In addition, the timing of initiation of conglomeratic sedimentation at a given section is a function of the spatial relationship between the section and the former axis of

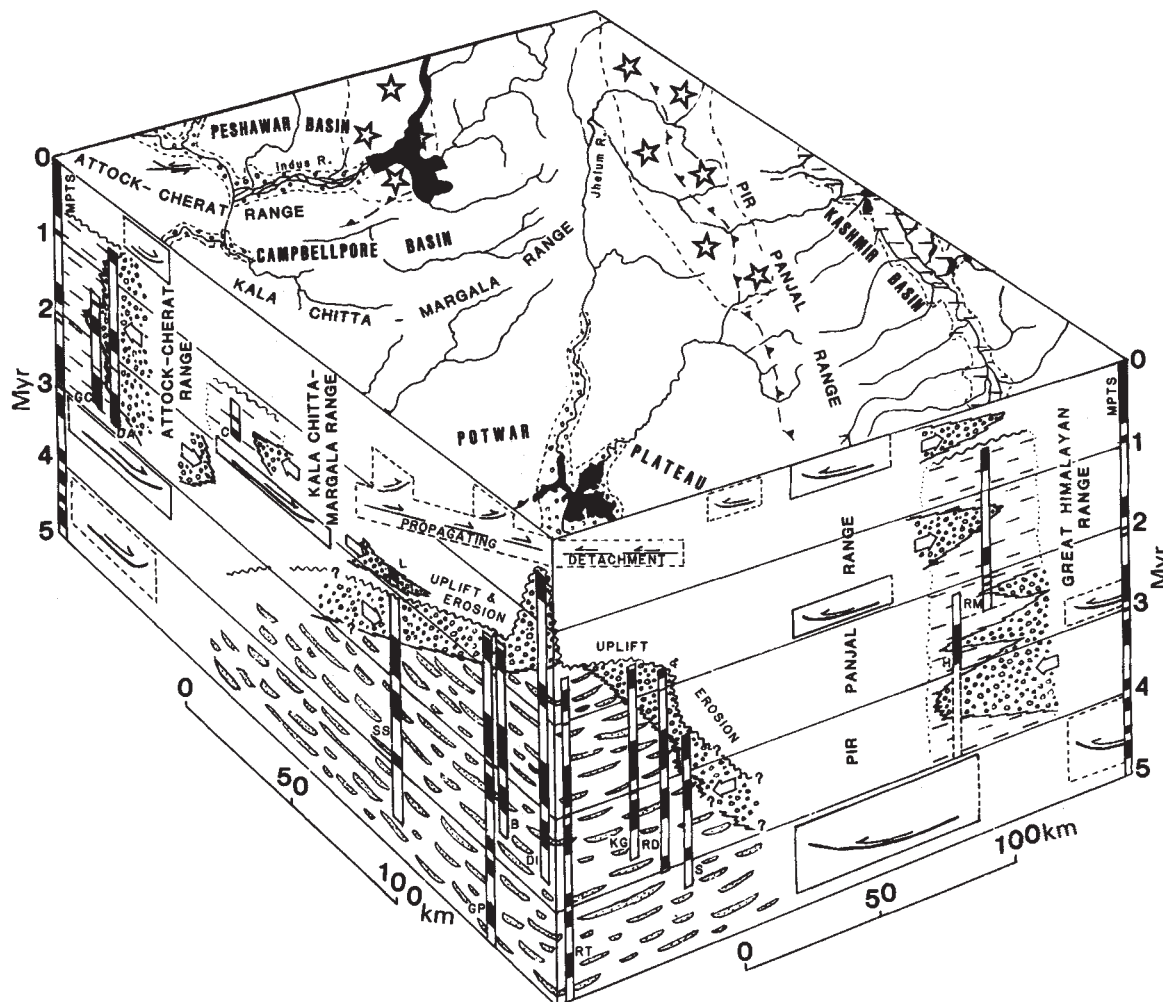


Fig. 6 Time-distance block diagram of the Himalayan foredeep and adjacent ranges in the vicinity of the North-west Syntaxis. The vertical dimension extends back to 5 Myr. The dated sequences described in this study and shown in Fig. 2 are depicted in their chronological range and are projected from their geographical location on to the transects defining the sides of the block. The abbreviations next to each magnetic column are as in Figs 1 and 2. Medial sections such as Sakrana (S) could be projected onto either face. The northeastern face is chosen here based on the affinities of the conglomerates at Sakrana with those from Rata-Dadial (RD) and Kas Guma (KG). For each of the stratigraphical sections, generalized depositional and facies relationships are shown in their proper chronological position (for example, the southward progradation of conglomerates from Sakrana across the eastern Potwar Plateau after 3 Myr). The intermontane basins are dominated by low-energy (largely lacustrine) facies punctuated by conglomeratic influxes. Two broad phases of molasse sedimentation across the Potwar Plateau are shown: a channel-and-floodplain interval succeeded by late-stage polymictic conglomerates. Large, open arrows indicate the prevailing palaeocurrent directions at different times. The boxes enclosing a thrust-fault symbol delineate both the spatial and chronological intervals over which the thrusts are interpreted to have been active. Dashed boxes show less well constrained periods of thrusting. On the left-hand panel, thrusting, folding and intermontane basin formation are seen to progress in a stepwise fashion from the Peshawar Basin to the south-east between 4 and 1 Myr ago. On the right-hand panel, three intervals of uplift in the vicinity of the Pir Panjal Range have defined the Kashmir Basin, controlled palaeocurrent patterns, and deformed the adjacent foredeep to the south-west. The surface of the block illustrates active, present-day processes. Deposition is largely restricted to the axial portions of the intermontane basins and narrow floodplains. Regions of high seismicity within 10–15 km of the surface²² are shown by stars. Presently- or recently-active faults that break the surface seem to be associated with shallow seismicity.

local fluvial deposition. Intraformational conglomerates first appear at Ganda Paik and Bangala (Fig. 1) about 2.0–1.9 Myr ago, synchronous with the inferred movement along the MBT. At the same time, decreasing rates of sedimentation are observed at Ganda Paik, Bangala, and, farther to the south, at Dina (Fig. 1), where extrabasinal conglomerates first appear around 1.6 Myr ago. The youngest conformable molasse strata in each section provide a limiting date on the cessation of sedimentation, as a wave of folding, uplift and erosion traversed the region. Thus, to the south-east of the Soan Syncline where deformation began around 2.1 Myr ago, the topmost conformable strata are progressively younger at Ganda Paik and Bangala (~1.6–1.5 Myr) and at Dina (~0.5 Myr). The youngest strata preserved farther south-east at Rohtas (Fig. 1) are ~1.5 Myr old. However, the presence of molasse strata as young as 0.4 Myr on the Rohtas anticline some 10–15 km farther south-west^{11,12} (BK, Fig. 1) suggests significant truncation of the Rohtas section.

Eastern limb of Jhelum Re-entrant

The Kashmir intermontane basin is separated from the Indo-Gangetic foredeep by the thrust-faulted Pir Panjal Range (Fig. 1). South of the Pir Panjal, the Siwalik molasse is well exposed in a series of thrust-cored anticlines where measured and dated stratigraphical sections record the history of fluvial systems flowing from the Himalayas. Although the detailed timing of tectonic events in the enclosing ranges and the bounding foredeep cannot be delineated as precisely as in the region to the west, three intervals of deformation can be discerned in both the intermontane sediments of Kashmir and the molasse sediments to the south.

In Kashmir, the basin-filling Karewa Formation^{19,21,33–36} is >1,300 m thick. Magnetostratigraphical sections from Hirpur and Romushi (Figs 1 and 2) indicate that Karewa sedimentation spans the interval from ~4 Myr to the present¹⁹. This deposition

began as a response to the initial emergence of the ancestral Pir Panjal Range ~4.5 Myr ago. Although molasse sedimentation across the Potwar region to the south continued uninterrupted during this interval¹³, major changes in clast lithologies in the molasse and in drainage patterns (from east-flowing to south-flowing)^{13,37} signal the concomitant onset of structural development of the Jhelum Re-entrant³⁸. In addition, for the first time in Upper Siwalik sediments, polymictic conglomerates appear in abundance, reaching Sakrana (Fig. 1) by 3.1 Myr ago and prograding southwards across Rata-Dadial (2.5 Myr) and Kas Guma (2.1 Myr). This onslaught of conglomeratic sedimentation appears to be a time-transgressive response to the initial uplift of the Pir Panjal Range.

Concurrently in Kashmir, palaeocurrent indicators¹⁹ suggest that pulses of uplift, presumably thrust-related, occurred along the northeastern margin of the basin until ~2 Myr ago. Subsequently, diminution of this thrusting³⁸ and accelerated uplift of the Pir Panjal Range redirected palaeocurrents towards the north-east. This interval of tectonism is synchronous with that previously described in the north-central Potwar and is recorded concurrently by a deceleration in molasse sedimentation rates at Rata-Dadial and Kas Guma (Fig. 1). This deceleration is likely to signal the onset of development of the thrust-cored anticlines in the foredeep in response to intensified thrusting and uplift to the north. Subsequently, progressive uplift and erosion spread southwards across the eastern Potwar, as evidenced by the youngest conformable strata at Sakrana (<2.7 Myr), Rata-Dadial (<1.8 Myr) and Kas Guma (<1.4 Myr). The youngest deformation in the Potwar is recorded in sections at Pabbi^{32,39}, Basawa Kas^{11,12} (P, BK; Fig. 1) and Dina, where erosion supplanted sedimentation <0.5–0.4 Myr ago. This third phase of tectonism is contemporaneous with the most recent interval of rapid uplift, totalling 1,300–3,000 m, of the Pir Panjal Range in southern Kashmir¹⁹.

Synthesis

The use of magnetostratigraphy in conjunction with fission-track dating provides an insight into the chronology of the late Cenozoic structural disruption of the northern margin of the Indo-Gangetic foredeep in northern Pakistan and India. Figure 5 illustrates the reconstructed depositional and tectonic conditions in 1-Myr increments between 4 Myr and the present. The time-space perspective diagram in Fig. 6 illustrates the concurrent evolution of both arms of the North-west Syntaxis.

Intermontane basin development commenced in Kashmir 4–5 Myr ago and progressed contemporaneously with major changes in palaeocurrents and provenance in the adjacent foredeep. Dated sequences in the Peshawar and Campbellpore intermontane basins and in the Soan Syncline delineate the sequential southward migration between ~3.0 and 1.8 Myr ago

of major thrust faulting, uplift, and the concurrent development of intermontane basins on the back side of the uplifted terranes. During this time interval, the locus of thrusting shifted some 50 km to the south. The duration of significant movement along the major thrusts (Attock, MBT) is envisaged as rather brief (<0.5 Myr). Contemporaneous deformation and erosion of sedimentary sequences in front of the thrusts often occurred at rapid rates ($\geq 15 \text{ mm yr}^{-1}$). Subsequent to the development of the imbricated, thrust-bounded ranges and basins in northern Pakistan and to a second pulse of uplift in Kashmir, the locus of major deformation continued migrating towards the south, where it caused folding, faulting and rotation of the Potwar Plateau^{13,32,40}, and propagation of the detachment surface to the Salt Range.

Whereas the initial development of the Kashmir and Peshwar Basins does not appear to be synchronous, a pulse of deformation associated with thrusting along the MBT is manifested across a broad region of the northwestern foredeep around 2–1.5 Myr ago. Development of the Campbellpore Basin, snapping shut of the Soan Syncline, and renewed uplift of the Pir Panjal Range were apparently contemporaneous with the disruption of molasse sedimentation across the Potwar Plateau as thrust-cored anticlines began to grow along the northern foredeep margin.

Conclusions

Because of the temporal resolution provided by magnetostratigraphical studies, the timing and duration of tectonic and sedimentary events in the northern Himalayan foredeep can now be reconstructed with greater detail than was previously possible. The chronological data emphasize the sporadic nature of tectonism in this collisional setting. Although the rate of convergence of the Indian subcontinent with Eurasia⁴¹ appears steady during the studied time interval, this detailed reconstruction of the northwestern Himalayan foredeep indicates that prolonged periods of quiescence and uniform sedimentation were punctuated by brief, intense intervals of deformation as the locus of thrust faulting encroached in a stepwise fashion on the adjacent foredeep. This sporadic tectonism may be regarded as the pulse of an orogenic process which, when integrated over time intervals in excess of 1 Myr seems to be continuous.

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Occurrence of a transposition from the X-chromosome long arm to the Y-chromosome short arm during human evolution

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DXYS1, a site showing greater than 99% DNA sequence homology between the human X and Y chromosomes, maps to the X long arm and to the Y short arm. In great apes, sequences homologous to DXYS1 are found only on the X chromosome. These findings suggest an X-Y transposition during human evolution.

It is widely held that the mammalian X and Y chromosomes are in part homologous as a consequence of their evolution from a completely homologous pair^{1,2}. Terminal pairing of the short arms of the human X and Y chromosomes during meiosis is taken as evidence of such partial homology^{1,3-8}. Here we demonstrate that human X-Y sequence homology is not limited to the pairing portions of the sex chromosomes. Moreover, X-Y homology at the locus *DXYS1* appears to be the result of a recent transposition from the X to the Y chromosome.

Chromosomal sublocalization of *DXYS1*

DXYS1 was the first site of single-copy DNA sequence homology to be found between the human X and Y chromosomes^{9,10}. A *TaqI* restriction fragment length polymorphism (RFLP)¹¹ at *DXYS1* consists of allelic X-linked fragments of 11 and 12 kilobases (kb) and a male-specific (Y-specific) 15-kb fragment. The complete association of the 15-kb fragment with the Y chromosome and the strictly X-linked inheritance of the 11- and 12-kb fragments imply that X-Y recombination does not normally occur (or is lethal) at *DXYS1*. Given the long-standing hypothesis that meiotic recombination between the pairing regions of X and Y chromosomes occurs frequently¹²,

we set out to determine whether *DXYS1* maps to the pairing regions of the sex chromosomes.

To localize *DXYS1* on the X and Y chromosomes, we carried out *in situ* hybridization of a probe defining *DXYS1* [pDP31, a subclone of probe 5 (Fig. 2a) into pBR322; ref. 9] to human metaphase chromosomes. As illustrated by the representative cell in Fig. 1a, pDP31 showed significant hybridization to the proximal portion of the long arm of the X chromosome and to the short arm of the Y chromosome. Of 72 cells hybridized at 100 ng ml⁻¹ probe DNA and exposed for 5-11 days, 47% exhibited grains on the proximal long arm of the X chromosome and/or on the short arm of the Y chromosome. These grains represented 20% of all label observed in the 72 cells. Experiments were then done to refine the sublocalization of *DXYS1* by hybridization of the ³H-labelled probe at a low concentration (12 ng ml⁻¹) followed by exposure for 46 days. Analysis of grains on labelled X and Y chromosomes (Fig. 1b) indicated sublocalization of *DXYS1* to Yp and to a region on the X that includes the lower half of band q13 and the upper half of q21 (most likely Xq13.2-q21.2).

To provide a second, independent sublocalization of *DXYS1* on the human X and Y chromosomes, genomic DNAs were

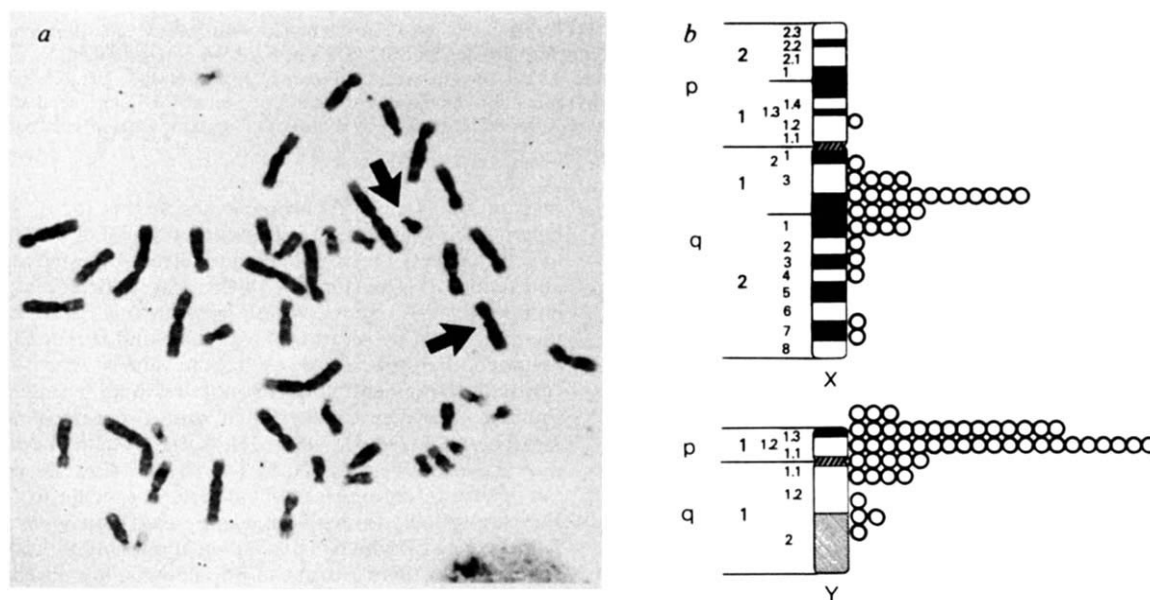
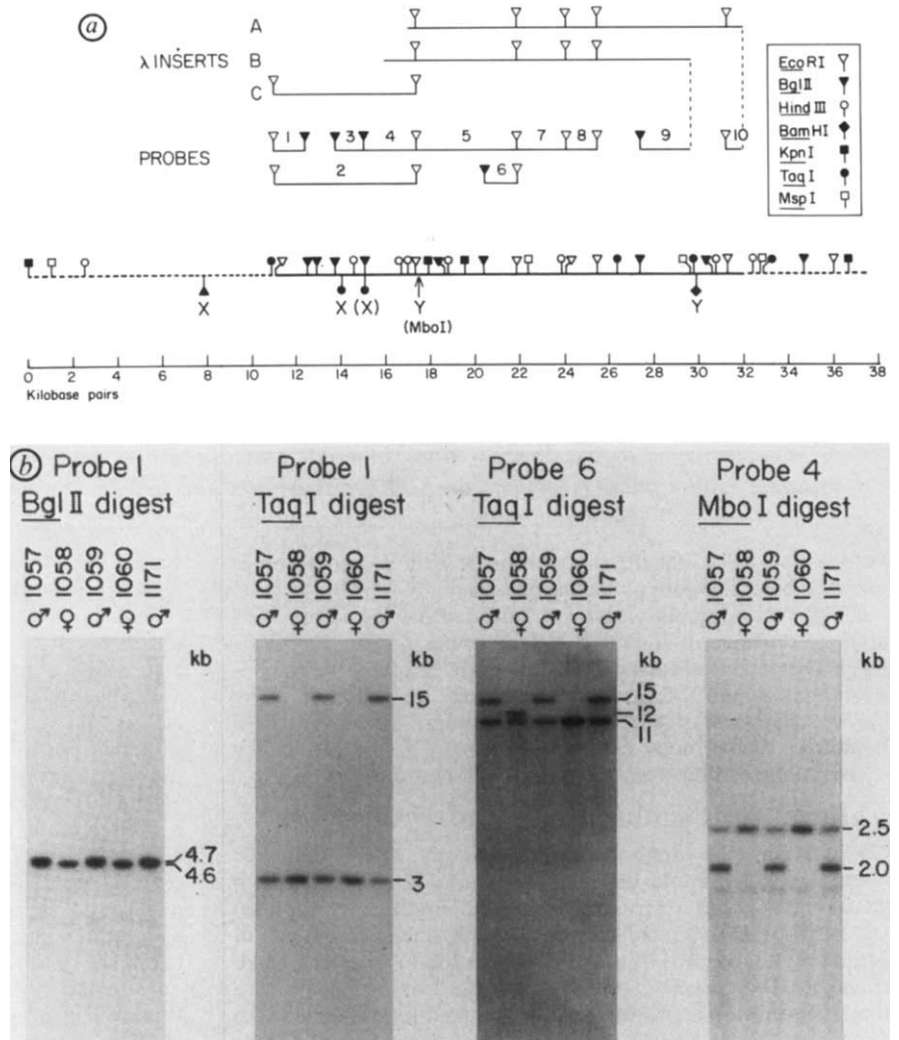


Fig. 1 Sublocalization of *DXYS1* in the human karyotype. *a*, Metaphase cell hybridized with ³H-labelled pDP31 (probe 5 in Fig. 2a subcloned into pBR322; ref. 9) illustrating typical labelling of the proximal portion of the long arm of the X chromosome and the short arm of the Y chromosome. *b*, Distribution of grains on metaphase (400-band stage) X and Y chromosomes from 51 labelled cells. pDP31 DNA was ³H-labelled by nick-translation and hybridized at low probe concentrations (12-100 ng ml⁻¹) to male mitotic preparations according to the method previously described³⁰.

Fig. 2 a, Restriction endonuclease mapping of 36 kb of the human X and Y chromosomes at *DXYS1*. At the top are shown the overlapping human DNA inserts of three recombinant λ phage: A, λ -rHs-3-1; B, λ -rHs-4813; and C, λ -rHs-X6.3. The locations of *Eco*RI restriction sites are indicated. Below the phage inserts are 10 restriction fragments purified from these inserts for use as hybridization probes; terminal restriction sites are indicated. Below the probes is shown the restriction map of the X and Y chromosomes at *DXYS1*. Restriction endonuclease cleavage sites common to the X and Y chromosomes are indicated by symbols above the line. Sites specific to either the X or the Y chromosome are indicated below the line. A Y-specific *Mbo*I site (see text) is shown. Sites within the 21-kb region (solid line) spanned by the three λ clones were mapped by direct analysis of those clones and confirmed by blot hybridization using probes 1–10. Sites outside the region (dotted line) were mapped by blot hybridization using probes 1 and 10. Every site was detected or confirmed by blot hybridization of DNAs from at least nine unrelated males and two unrelated females (nine Y chromosomes and 13 X chromosomes). We have previously reported a Y-linked *Msp*I/*Bgl*II restriction fragment length polymorphism at *DXYS1* (ref. 9); this polymorphism is the result of a tandem duplication on the Y chromosome in some males (D.C.P., in preparation). The map of the Y chromosome shown here is that of the nonduplicated form. No new RFLPs were detected during this study. **b**, Differential patterns of hybridization of human male and female DNAs with DNA probes for *DXYS1*. These hybridizations detected restriction sites specific to either the X or the Y chromosome. **Methods:** Recombinant phage λ -rHs-4813, purified from a human genomic library³¹, was the original source of hybridization probes for *DXYS1* (ref. 9). Recombinant phages λ -rHs-3-1 and λ -rHs-X6.3 derive from the same genomic library³¹ and from an X-specific library³², respectively. These two phage were identified by plaque hybridization screening³³ using DNA probes purified from the human insert of λ -rHs-4813. Some of these restriction fragments were subcloned into plasmid pBR322 or an abbreviated derivative (pDP322; D.C.P., unpublished) before use as hybridization probes. Human genomic DNAs were prepared from peripheral leukocytes by the method of Kunkel *et al.*¹³. DNA samples were digested with restriction endonuclease, electrophoresed on 0.75% agarose gels, and transferred³⁴ to AMF Zetapore paper. Restriction fragments purified from phage λ -rHs-3-1, λ -rHs-4813 and λ -rHs-X6.3 were labelled with ³²P by nick-translation³⁵ and hybridized overnight to the filter-bound genomic DNAs at 47 °C in 50% formamide, 5 $\times$ SSC (1 $\times$ SSC = 0.15M NaCl, 15 mM Na citrate pH 7.4), 1 $\times$ Denhardt's (0.02% Ficoll 400, 0.02% polyvinyl pyrrolidone, 0.02% bovine serum albumin), 20 mM NaPO₄ pH 6.6, 50 μ g ml⁻¹ denatured salmon sperm DNA, and 10% dextran sulphate. Following hybridization, filters were washed three times for 15 min each at 55–60 °C in 0.1 $\times$ SSC, 0.1% SDS and exposed at -70 °C for 1–7 days with Kodak XAR-5 film backed by a DuPont Lightning-Plus intensifying screen.



obtained from three rodent-human cell hybrids (characterized by G. Bruns, unpublished results) containing portions of the human X chromosome and from three humans whose cells contain portions of the Y chromosome (ref. 13 and K. Smith and collaborators, unpublished results). Gel transfers of *Taq*I digests of these DNAs were hybridized with *DXYS1* probe 5 (see Fig. 2a) and scored for the presence or absence of the X- and Y-specific *Taq*I fragments detected by probe 5. The results of these studies map *DXYS1* to Xq1-q22 and to Ycen-pter (data not shown). In addition, *DXYS1* probe 9 (Fig. 2a) was applied to the dosage panel used by Kunkel *et al.*¹⁴ to assign DNA segments to regions of the human X chromosome. By this method, *DXYS1* was mapped to Xq13-q24 (data not shown). These results are consistent with the assignment of *DXYS1* to Xq13-q21 and Yp by *in situ* hybridization.

X-Y restriction mapping

The homology between the X and Y chromosomes at *DXYS1* extends for at least 28 kb⁹. In order to quantitate further the X-Y homology at *DXYS1*, we made a comparative restriction

map of the X and Y chromosomes at this locus. A series of neighbouring single-copy restriction fragments were hybridized to gel transfers of restriction endonuclease-digested human male and female DNAs (Fig. 2). In this way, a stretch of 36 kb was mapped in this region. Where no differences were detected in the patterns of hybridization with male and female DNA, it was assumed that the X and Y chromosomes share a common restriction fragment. Indeed, most restriction fragments were found to be identical on the X and Y chromosomes. Of 40 *Eco*RI, *Bgl*II, *Hind*III, *Bam*HI, *Kpn*I, *Taq*I and *Msp*I restriction sites that we mapped at *DXYS1*, 36 sites are common to the X and Y chromosomes, one site is specific to the Y, two sites are specific to the X, and one (*Taq*I) site gives rise to the X-linked RFLP which is present on about half of the X chromosomes in Northern European populations, but absent from the Y chromosome. These results establish that the X and Y chromosomes are homologous over an expanse of at least 36 kb at *DXYS1*. We have not detected the termination of this X-Y sequence homology.

Analysis of this region with the restriction enzyme *Mbo*I yields similar results. Of 11 *Mbo*I restriction fragments observed over

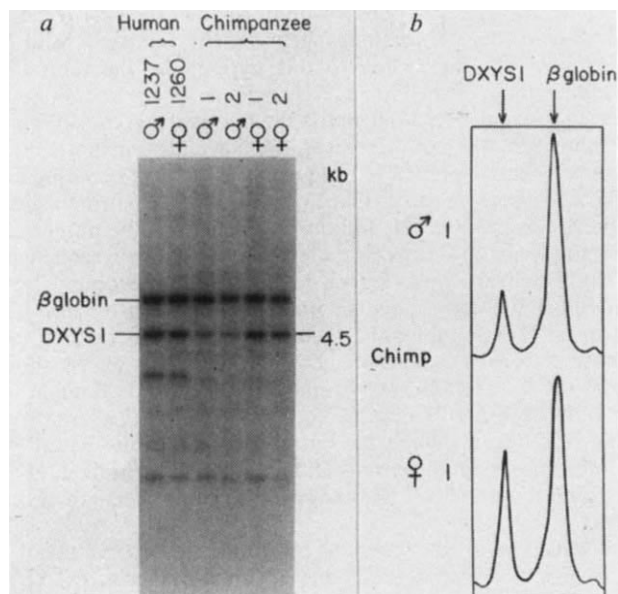


Fig. 3 Male-female dosage at a *DXYS1*-homologous locus in chimpanzee. *a*, Hybridization of *EcoRI*-digested human and chimpanzee male and female DNAs to probes for *DXYS1* and β -globin. Genomic human and chimpanzee DNAs were digested with the restriction endonuclease *EcoRI*, blotted, and hybridized as in Fig. 2*b* with ^{32}P -labelled probe 5 (Fig. 2*a*) and plasmid $\pi\text{SV}\beta$ (containing 3.7 kb of human genomic sequence from the β -globin locus; ref. 36). Probe detects a 4.5-kb human genomic *EcoRI* fragment common to the human X and Y chromosomes (Fig. 2*a*). It is also homologous to a 4.5-kb *EcoRI* fragment in chimpanzee. Plasmid $\pi\text{SV}\beta$ detects a 5.3-kb *EcoRI* fragment in both human and chimpanzee. In the human, this *EcoRI* fragment contains most of the β -globin gene and derives from chromosome 11. $\pi\text{SV}\beta$ also hybridizes to two smaller *EcoRI* fragments in both human and chimpanzee (in man, these are known to correspond to the remainder of the β -globin locus and to the δ -globin locus). *b*, Densitometric tracings of portions of two lanes (chimpanzee male no. 1 and female no. 1) of an autoradiogram produced without an intensifying screen but otherwise identical to that in *a*.

~22 kb at *DXYS1*, nine are common to the X and Y chromosomes, one is specific to the Y chromosome, and one is specific to the X chromosome. Thus, of at least 12 *MboI* sites represented by these fragments, one site is specific to the Y (Fig. 2*a*), and all other sites are common to the X and Y chromosomes. The results of several hybridization experiments that revealed restriction sites specific to either the X or the Y chromosome are shown in Fig. 2*b*.

Sequence divergence

The fraction of nucleotides at which a pair of homologous DNAs differ can be estimated by comparing the restriction maps of the two sequences. Given the comparative X-Y restriction map of *DXYS1* (Fig. 2*a*), we set out to determine the fraction of nucleotides at which the X and Y chromosomes differ in this region. This fraction was estimated using the iterative 'nucleotide counting' method of Nei and Tajima¹⁵. In using this method, we assume that restriction cleavage sites are created or eliminated solely by substitutions of base pairs. The calculation involves only those mapped restriction sites which fall within the 21 kb at *DXYS1* which we have cloned, and which is mapped in its entirety for seven enzymes (Fig. 2*a*). The data on *MboI* fragments are not included in the calculation as they do not represent mapped sites. Drawing on data for the seven other enzymes, we estimate that the human X and Y chromosomes differ at $0.83 \pm 0.54\%$ of their nucleotides at *DXYS1* (Table 1). It has been argued that CpG nucleotide dimers (such as occur within the recognition sequences of the enzymes *TaqI* and *MspI*) are remarkably prone to mutation in humans¹⁶. If the *TaqI* and

Table 1 Calculations of X-Y sequence divergence at *DXYS1*

Distribution of restriction sites on the X and Y chromosomes			Distribution of restriction sites on the X and Y chromosomes		
6-base enzymes	m_X	m_Y	4-base enzymes	m_X	m_Y
<i>EcoRI</i>	6	6	<i>TaqI</i>	3.5	2
<i>BglII</i>	8	8	<i>MspI</i>	2	2
<i>HindIII</i>	6	6	Total	5.5	4
<i>BamHI</i>	0	1			
<i>KpnI</i>	2	2	$\bar{m} = 4.75$		
Total	22	23			

$\bar{m} = 22.5$

Combined 6- and 4-base enzyme data: $d = 0.0083 \pm 0.0054$

6-base enzyme data only: $d = 0.0037 \pm 0.0038$

The fraction of nucleotides at which the X and Y chromosomes differ at *DXYS1* was estimated using data drawn from the X-Y comparative restriction map of Fig. 2*a* and formulae of Nei and Tajima¹⁵:

$$d = d_1 \frac{\sum_i r_i (\bar{m}_i - m_{XY}) / [(1 - d_1)^i] \{2 - (1 - d_1)^i\}}{\sum_i r_i \bar{m}_i / [2 - (1 - d_1)^i]}$$

where $d_1 = 1 - \hat{S}^{1/r}$,

$$\hat{S} = \frac{2m_{XY}}{m_X + m_Y}$$

i refers to the i th type of enzyme (in this case there are two types, 4-base and 6-base enzymes),

$$\bar{m} = (m_X + m_Y) / 2$$

and where the standard error of d is

$$s_d = \left(\frac{1}{\sum_i \frac{2r_i^2 \bar{m}_i S_i}{(2 - S_i)(1 - S_i)}} \right)^{1/2}$$

where

$$S_i = e^{-r_i d}$$

where d is the estimated fraction of nucleotides at which X and Y differ; r_i , the number of base pairs recognized by a given restriction enzyme (in this case, 6 or 4); m_X and m_Y , the number of restriction sites on, respectively, the X and Y chromosomes; m_{XY} , the number of restriction sites shared by the X and Y chromosomes. Note that the polymorphic *TaqI* site on the X chromosome contributes 0.5 to the value of m_X for *TaqI*.

MspI data are eliminated from the calculation of X-Y sequence divergence, the estimate of that divergence falls to $0.37 \pm 0.38\%$.

Homologous sequences in great apes

Our estimate of X-Y sequence divergence at *DXYS1* ($0.37 \pm 0.38\%$) is lower than estimates of the divergence of the human and chimpanzee genomes as a whole ($1-3\%$)¹⁷⁻²⁰. Assuming that the X and Y chromosomes do not evolve more slowly than the autosomes, this suggests one of two possibilities. First, *DXYS1* could represent an evolutionarily ancient homology between the X and Y chromosomes and some mechanism could retard or repair X-Y divergence at that locus. Alternatively, the presence of *DXYS1* sequences on both the X and Y chromosomes might be a phenomenon limited to recent evolutionary times. According to this hypothesis, *DXYS1* sequences have been present on both the X and Y chromosomes only for a period comparable to or shorter than that since humans diverged from chimpanzees.

To distinguish between the two possibilities, genomic DNAs from the great apes were examined for sequences homologous to *DXYS1*. If *DXYS1* represents an ancient homology between the X and Y chromosomes, one would expect to find *DXYS1*-homologous sequences on both the X and Y chromosomes in all the great apes. However, if X-Y homology at *DXYS1* is of recent origin, one would expect to find *DXYS1*-homologous sequences on either the X or Y chromosome, but not on both.

Filter hybridization experiments revealed that the great apes have *DXYS1*-homologous sequences only on their X chromosomes. Gel transfers of *EcoRI* digests of human and ape DNAs were hybridized with probe 5 (Fig. 2*a*) at moderately high stringency as described in Fig. 2 legend. At least one male and one female of each of three ape species (chimpanzee, gorilla

Table 2 Dosage of *DXYS1*-homologous sequences in male and female ape DNAs

	Sex	Autosomal standard:	X-linked standard:	No. of <i>DXYS1</i> copies per cell
		Ratio of areas <i>DXYS1</i> / β -globin	Ratio of areas <i>DXYS1</i> / <i>DXS9</i>	
Chimpanzee	M	0.30 (0.5)	0.83 (0.9)	1
	M	0.33 (0.6)	0.88 (1.0)	1
	F	0.59 (1.0*)	0.92 (1.0*)	2
	F	0.60 (1.0)	0.89 (1.0)	2
Gorilla	M	0.36 (0.6)	1.06 (1.1)	1
	F	0.62 (1.0*)	0.96 (1.0*)	2
	F	0.75 (1.2)	0.97 (1.0)	2
Orangutan	M	—	1.17 (1.2)	1
	F	—	1.00 (1.0*)	2
Human	M	0.60 (1.1)	1.95 (2.4)	2
	F	0.55 (1.0*)	0.83 (1.0*)	2

Genomic human, chimpanzee, gorilla and orangutan DNAs were digested with *EcoRI*, blotted and hybridized with probes for *DXYS1* and β -globin or *DXYS1* and *DXS9* (RC8, ref. 21) as described in Fig. 3 legend. To ensure a linear relationship of filter-bound radioactivity to absorbance on the autoradiogram, no intensifying screen was used. As in Fig. 3b, densitometric scans of the autoradiograms were made. The areas under the peaks corresponding to *DXYS1* and β -globin or *DXYS1* and *DXS9* were integrated, and, for each species, the ratios of these areas were normalized to the female, indicated by an asterisk. (*DXYS1*/ β -globin ratios could not be calculated for the orangutans because of poor separation of the *DXYS1* and β -globin peaks.) The number of copies of *DXYS1* per cell was calculated assuming that females have two copies per cell of both β -globin and *DXS9* and that males have two copies per cell of β -globin and one of *DXS9*.

and orangutan) were examined. In humans, probe 5 detects a 4.5-kb *EcoRI* fragment that is common to the X and Y chromosomes. Thus there are two copies per cell in both males and females. All ape DNAs contained sequences homologous to *DXYS1* (for example, Fig. 3a). In the gorilla and chimpanzee, probe 5 detects a 4.5-kb *EcoRI* fragment, and in the orangutan, probe 5 detects two *EcoRI* fragments of 3.4 and 5.3 kb. Unlike the human, however, in all three ape species the dosage of *DXYS1*-homologous sequences is a function of sex. As judged using an autosomal (β -globin) or X-linked (*DXS9*, probe RC8; ref. 21) reference standard, in each of the three ape species, females contain twice as many copies of the *DXYS1*-homologous sequence as do males (Table 2, Fig. 3); no male-specific *EcoRI* (or *TaqI*, data not shown) fragment was found in any of the apes. The most economical interpretation of these findings is that *DXYS1*-homologous sequences occur only on the X chromosome among the great apes. Thus, it seems that X-Y homology at *DXYS1* is of recent origin and, among extant species, is found only in man.

Conclusions

Homology of single-copy DNA sequences on mammalian X and Y chromosomes was first reported at the human locus *DXYS1* (ref. 9), and there is now increasing evidence of substantial homology of single-copy sequences of no known function on the human X and Y chromosomes²². However, although a monoclonal antibody elicited by an antigen (12E7) encoded by the human X chromosome cross-reacts with an antigen coded for by the human Y chromosome²³, there is little rigorous evidence of homologous functional genes on the X and Y chromosomes of any mammal.

Our mapping of *DXYS1* to Xq13-q21 is consistent with the findings of other workers. J. Weissenbach (personal communication) has, by means of a different panel of rodent-human cell hybrids containing portions of the human X chromosome, assigned *DXYS1* to Xq12-q22. Drayna *et al.*²⁴ have recently demonstrated a genetic distance of about 12 centimorgans between *DXYS1* and *DXS17* (S21), which in turn has been mapped to Xq21-q22.

The findings reported here are not consistent with a model that limits X-Y sequence homology to the short arms of the X and Y chromosomes. Terminal portions of the short arms of the X and Y chromosomes pair during meiosis in human sper-

matogenesis³⁻⁷, and this observation provided the basis for the prevailing model of homology between the human X and Y chromosomes^{1,8}. According to this hypothesis, the terminal, pairing portions of the X and Y chromosomes are homologous at the DNA sequence level, while the non-pairing, or 'differential', portions are not. However, we have demonstrated the presence of single-copy DNA sequences (*DXYS1*) homologous to the Y chromosome at Xq13-q21, far outside the pairing region of the X chromosome. In addition, there seem to be numerous other single- or low-copy number²² and repetitive²⁵ sequences on Xq which are homologous to the Y chromosome. Thus, although single-copy sequence homology between Xp and Yp may exist, X-Y homologous sequences are clearly not limited to those regions. Furthermore, *DXYS1* sequences on the short arm of the Y chromosome, virtually all of which is thought to pair with the X (ref. 26), have no homologue on the short arm of the X. Thus, although the pairing regions of the X and Y chromosomes may be partially homologous, it is unlikely that there is continuous X-Y homology throughout the length of the pairing region.

We have previously reported⁹ our failure to observe meiotic recombination between the X and Y chromosomes at *DXYS1*. Given the location of *DXYS1* at Xq13-q21, it is now clear that single reciprocal cross-overs between the X and Y at *DXYS1* would destroy the integrity of both the X and Y chromosomes.

It is widely accepted that the mammalian X and Y chromosomes evolved from a completely homologous pair². It has been assumed, as a corollary, that if limited homology of the human X and Y chromosomes exists, the homology should be ancient in an evolutionary sense¹. The findings reported here demonstrate that this need not be the case. Among extant species, *DXYS1*-homologous sequences apparently occur on both the X and Y chromosomes only in man; in chimpanzee, gorilla and orangutan, *DXYS1*-homologous sequences are found only on the X chromosome. Thus, the simplest explanation is that *DXYS1*-homologous sequences were transposed from the X chromosome to the Y chromosome after the human line diverged. (To argue that the common ancestor of all four species had *DXYS1*-homologous sequences on both X and Y chromosomes, one must appeal to the loss of *DXYS1*-homologous sequences from the Y chromosome three times during evolution.) This hypothesis is consistent with the finding that the divergence of human X and Y DNA sequences at *DXYS1* is somewhat less than the divergence of the human genome from that of its closest relative, the chimpanzee. It is estimated that the human and chimpanzee lines diverged between 4 and 8 Myr ago^{20,27,28}. Thus, *DXYS1* sequences have been present on both the X and Y chromosomes for less than—perhaps much less than—8 Myr.

The full extent of the X-to-Y transposition that occurred at *DXYS1* during human evolution is unknown, but it is clear that at least 36 kb of DNA was involved. To our knowledge, this X-to-Y transposition has not been detected by comparative light microscopic examination of banded human and ape chromosomes²⁹. The recent finding^{22,25} of other human X-Y homologous sequences on Xq suggests either that the transposition was much larger than 36 kb or that it was in some way directed by preexisting X-Y homologies.

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Correlation between segmental mobility and the location of antigenic determinants in proteins

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Most continuous antigenic determinants of tobacco mosaic virus protein (TMVP), myoglobin and lysozyme correspond to those surface regions in the protein structure, as determined by X-ray crystallography, which possess a run of high-temperature factors along the polypeptide backbone, that is, a high segmental mobility. The mobility of an antigenic determinant may make it easier to adjust to a pre-existing antibody site not fashioned to fit the exact geometry of a protein. The correlation found between temperature factors and antigenicity is better than that between hydrophilicity and antigenicity.

THE antigenic reactivity of proteins resides in restricted parts of the molecule known as antigenic determinants or epitopes. Antigenic determinants represent the accessible patches on the surface of a native protein that interact with the binding sites of antibody molecules. Epitopes made up of a continuous sequence of amino acid residues are called 'continuous determinants' while 'discontinuous determinants' consist of residues that are not contiguous in the primary structure but which are brought together by the folding of the polypeptide chain¹. The continuous epitopes comprise five to eight residues and their uniform size in different proteins reflects the constant size of the complementary antibody combining sites.

The localization of continuous determinants in proteins is based on measurements of the antigenic reactivity of natural or synthetic peptides of the molecule^{2,3}. Usually, the fragments are tested for their ability to inhibit the reaction between antibodies and the whole antigen, and very high molar ratios of peptide to intact antigen are often required to achieve significant inhibition; this is usually explained by assuming that peptides exist in solution in a variety of random conformations and that only the rare conformations which approximate to that found in the native protein will bind to the antibody^{4,5}. Both the N- and C-termini of different proteins often show higher than average antigenic activity^{6–14}, presumably because they are located at the surface and have a high relative flexibility¹⁵.

In the last few years, the importance of the internal dynamics of protein molecules in relation to function has been emphasized^{16–18}. This appreciation prompted us to examine whether there is a more general link between the local mobility of short segments of proteins and the antigenic structure. For this study we selected three proteins: the coat protein of tobacco mosaic virus (TMVP), myoglobin and lysozyme, which have been

studied extensively by X-ray crystallography^{19–21}. Present refinement methods^{22,23} give not only precise atomic coordinates but also atomic temperature factors (*B* values). The temperature factor represents the mean-square displacement of each atom and, when plotted against residue number, provides a graphic image of the degree of mobility existing along the polypeptide chain. Such plots were used to examine the possible correlation between the mobile segments of these proteins and the locations of their epitopes.

Tobacco mosaic virus protein

The antigenic properties of the coat protein of tobacco mosaic virus have been extensively studied by several groups²⁴. Milton and Van Regenmortel²⁵ originally reported that TMVP possesses five continuous antigenic determinants situated in tryptic peptides I, IV, VIII and XII. Peptide I contains two epitopes located in residues 1–10 and 34–39. The epitope in peptide VIII has been studied extensively by Benjamini²⁶, who located it in residues 105–112, although some residual activity is also found in the shorter pentapeptide, 108–112. Two additional antigenic determinants were recently identified¹⁰ in residues 55–61 and 80–90, by comparing the inhibitory activity of tryptic peptides II, III (42–61) and VI (72–90) from three strains of the virus. Three peptides corresponding to residues 72–77, 129–134 and 142–147 that were synthesized because they correspond to accessible regions on the surface of the TMVP molecule were found to have no significant (that is, above the 10% level) antigenic activity in enzyme-linked immunosorbent assay (ELISA) inhibition tests.

Thus, the many studies of the antigenic structure of TMV^{10,26,27} have so far revealed only seven continuous epitopes in the protein. The immunoassays were carried out at very low

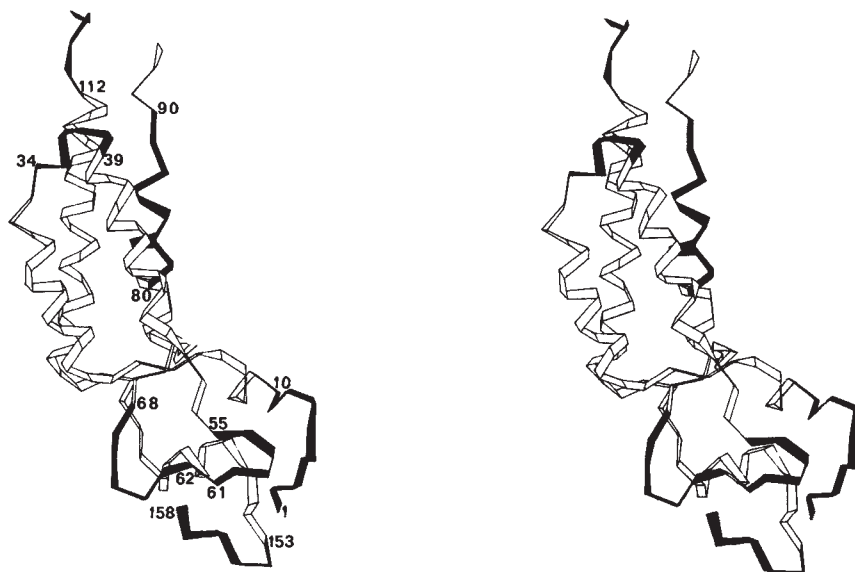


Fig. 1 Stereo drawing of the α -carbon backbone of the TMVP subunit at the present stage of refinement of the protein disk (A-ring). All residues (1–158) are shown except for 94–106, which are omitted because this region of the polypeptide has no specific conformation in the disk. The locations of the seven continuous antigenic determinants are indicated by solid lines.

concentrations where the protein is almost totally dissociated into monomers. The presence of very small amounts of other species would, in any case, not influence the measured degree of inhibition.

The seven epitopes are situated in the vicinity of residues 1–10, 34–39, 55–61, 62–68, 80–90, 105–112 and 153–158. Their positions in the TMVP molecule are shown in Fig. 1. The starting model of the TMVP subunit as seen in the protein disk at 2.8 Å resolution¹⁹ has been subjected to constrained crystallographic refinement²² to produce these coordinates and temperature factors (A.M. and A.C.B., in preparation). When the positions of the epitopes of TMVP were superimposed on a plot of the temperature factors of main-chain atoms in each residue (Fig. 2), it was found that all seven antigenic regions correspond to local maxima of the *B* value. Furthermore, the positions of six of the seven epitopes correlate with the major peaks, whereas only one epitope corresponds to a minor peak (residues 55–61); the lack of mobility in this seventh epitope can perhaps be explained by the fact that, in the crystal, residues 55–61 are constrained, in both layers of the TMV protein disk, by strong intermolecular contacts involving hydrogen bonding, hence they show a lower *B* value than would occur in an isolated monomer. A similar plot of the overall temperature factor for each residue (thus including the side-chain atoms) displays additional peaks which do not correspond to observed antigenic determinants. Thus, side-chain mobility does not correlate with antigenicity whereas that in the main chain does.

It is well known that the N- and C-termini of many proteins are flexible and accessible on the molecular surface¹⁵. In several cases (reviewed in 28, 29) the termini are known to be antigenic determinants (for example, the carboxy-terminal of histone H3 (ref. 9), TMVP¹⁰, simian virus 40 T-antigen³⁰). However, we did not expect that all the other known epitopes of TMVP would also belong to regions having higher than average flexibility. The three antigenically inactive peptides (residues 72–77, 129–134 and 142–147) lie in surface regions but do not have *B* values significantly different from the mean. This last finding shows that the correlation in Fig. 2 is not based solely on the high surface accessibility of these regions.

The locations of the seven TMVP epitopes were also superimposed on a plot (Fig. 3) of the hydrophilicity values of the protein residues calculated according to Hopp and Woods^{31,32}. These plots have been used to predict positions of epitopes in proteins for which only the sequence is known^{32–34}; their predictive value stems from the fact that hydrophilic residues lie predominantly at the surface and are therefore more likely to belong to epitopes. Although Fig. 3 shows a good correlation between antigenicity and some of the hydrophilicity peaks (for example, residues 62–68), several of the maxima correspond to

regions with no proven antigenic activity, and the agreement with hydrophilicity is thus less striking than that with *B* values.

Myoglobin

The antigenic structure of myoglobin has been studied extensively by Atassi and collaborators³⁵, who identified five epitopes in residues 15–22, 56–62, 94–99, 113–119 and 145–151. Although it was claimed that these five sequences accounted for all the antigenic properties of myoglobin³⁶, subsequent work showed that additional continuous epitopes are present in regions 1–6, 22–55, 72–89 and 121–127 (refs 37–40).

The temperature factors plotted in Fig. 4a are taken from the restrained least-squares refinement²² of oxymyoglobin at 1.6 Å resolution²⁰ using diffraction data collected at –12 °C. The *B* values are, in general, low in helical regions and high at bends and chain termini, but it is clear that in myoglobin the deviations from the mean are small²⁰ and few distinctive peaks are present (Fig. 4a). This observation is related to the high helix content and compact nature of the protein, which has no long flexible

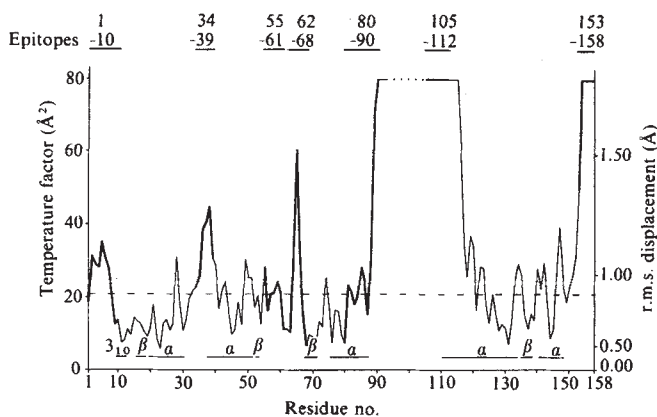


Fig. 2 The mean temperature factor of the main-chain atoms in each residue plotted against residue number for the TMVP disk. These temperature factors are from the constrained crystallographic refinement²² of the disk of TMVP at 2.8 Å resolution. Strict 17-fold non-crystallographic symmetry constraints were applied throughout the refinement. Temperature factors for the A-ring are shown here although those for the B-ring are essentially the same (A.M. and A.C.B., in preparation). The mean value of the temperature factor (omitting residues for which *B* > 80) is shown by a dashed line. The locations of the seven continuous antigenic determinants are indicated by thick lines. Regions of secondary structure are denoted α , β , γ . The r.m.s. value of the displacement is calculated from $U^2 = U_x^2 + U_y^2 + U_z^2 = 3B/8\pi^2$, where *U* is the total displacement and *U_x*, *U_y*, *U_z* are the components.

loops. The positions of six of the nine epitopes correlate with local maxima; however, one epitope (residues 56–62 at the tight corner between helices D and E) corresponds to a minimum. No conclusion can be made about the epitopes in peptides 22–55 and 72–89 (ref. 40) because of the uncertainty in their location.

Lysozyme

The antigenic structure of lysozyme has been studied by Atassi and colleagues⁴¹ who identified three discontinuous epitopes centred around the disulphide bridges 30–115, 76–94 and 6–127. It was claimed that these three epitopes account for all the antigenicity of lysozyme⁴², although other investigators have shown that the loop formed by the residues lying between the disulphide bridge at 64–80 (refs 43–46) and also residues 38–54 possess antigenic activity⁴⁷. The antigenic reactivity of the loop depends on the preservation of the disulphide bridge.

Figure 4b shows the variation of the temperature factor along the polypeptide chain of lysozyme²¹. The mean main-chain temperature factors are from the restrained least-squares refinement²³ of the tetragonal hen egg white lysozyme (HEWL) at 2 Å resolution. Three peaks are seen in Fig. 4b around residues 47, 70 and 101. The peaks at 47 and 70 correlate with the known epitopes, but are not found in a corresponding plot for the orthorhombic form of HEWL, where these residues are involved in molecular contacts within the crystal²¹. The third peak, 99–102 (which corresponds to one tip of the active site), is found in both forms of HEWL²¹ and also in human lysozyme²⁰. No antigenic activity has been reported to involve these residues, but there is a discontinuous epitope nearby on the sides of the active site cleft, which, as noted by Artymiuk *et al.*¹⁸, lies in a region of relatively high flexibility. We have isolated, by HPLC, a tryptic peptide that encompasses the region 99–102, but found that it possessed no antigenic reactivity in ELISA inhibition tests¹¹.

Conformational dependence of epitopes

Several opposing views of the nature of protein epitopes have emerged recently⁴⁸. In one picture, proteins contain only a small number of discrete antigenic regions situated in highly accessible surface locations^{36,42}. The presence of antigenic reactivity when these parts are excised from the intact molecule is then ascribed to the preservation of specific conformational features. That the antigenic reactivity of proteins is dependent on the native conformation is corroborated by the finding that there is usually very little cross-reactivity between the native and denatured forms of the molecule.

From studies of the immunological cross-reactivity between related proteins, a contrasting view has emerged—that virtually the entire accessible surface of a protein can combine with appropriate antibodies^{49,50}. Consequently a considerable pro-

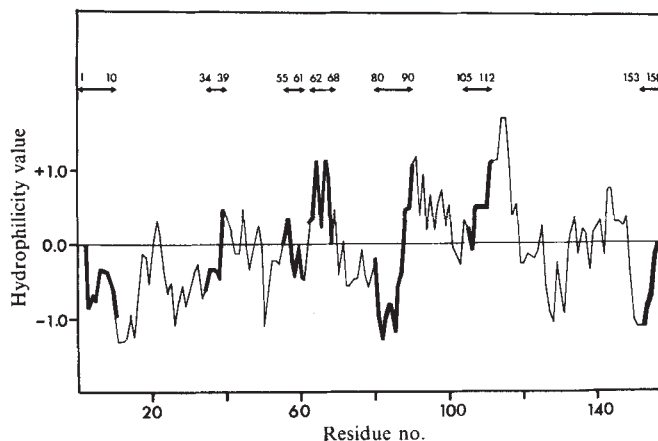


Fig. 3 The hydrophilicity value of each residue of TMVP (calculated after Hopp and Woods³¹) plotted against residue number, after averaging the value over six residues. The locations of the seven continuous epitopes are indicated by thick lines.

portion of the antigenic determinants of globular proteins must be discontinuous in character as only a small part of the surface of a globular protein is made up of linear arrays of residues^{40,48}. Indeed, attempts to locate protein epitopes by using monoclonal antibodies directed against the native molecule have led to the identification of mainly discontinuous epitopes^{51–54}. It seems that as monoclonal antibodies recognize a small sample of all antigenic specificities, they are more likely to be directed against the numerous surface patches of a protein made up of residues distant in the sequence, rather than against continuous determinants.

Clearly, discontinuous epitopes cannot be studied with antibodies specific for short linear peptides and cannot be identified in linear plots of flexibility along the protein chain. Although the correlation we have demonstrated between segmental mobility and the location of epitopes pertains only to continuous epitopes, the notion of flexibility in epitopes is of considerable interest in the current debate on what constitutes an antigenic determinant.

The classical method of localizing epitopes by measuring the antigenic reactivity of cleaved fragments of the protein with anti-protein antibodies is now being increasingly replaced by an analysis of the specificity of antibodies raised against various fragments. It was originally believed that antibodies reactive with the native protein would be produced only if the fragment used for immunization closely reproduced the tertiary conformation of the protein. However, recent work shows that many

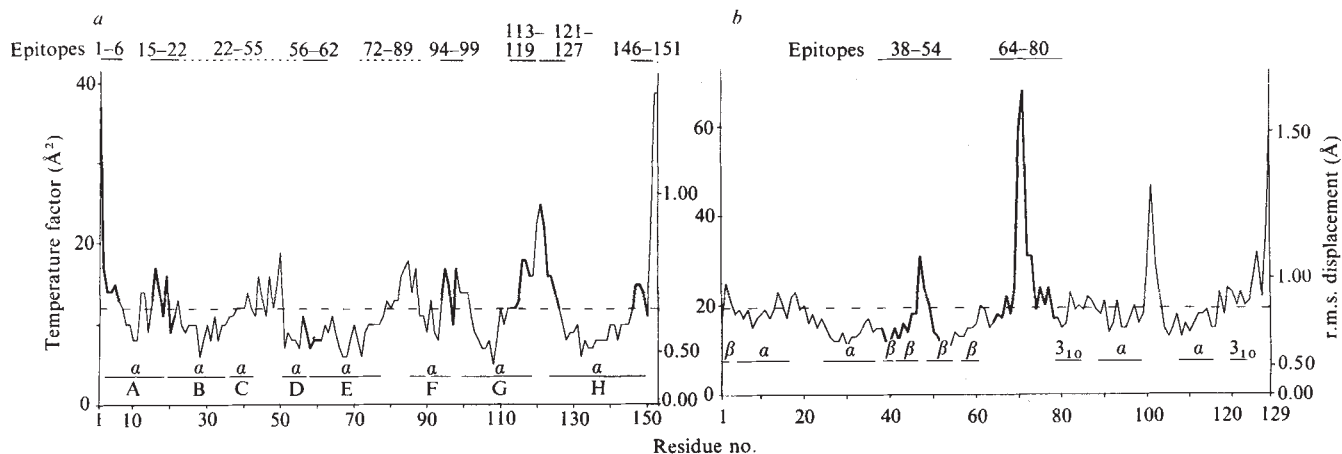


Fig. 4 The mean temperature factor of main-chain atoms plotted against residue number for: a, oxymyoglobin (redrawn after ref. 20) and b, lysozyme (redrawn after ref. 21). The mean temperature factor is shown by a dashed line. The first and last residue numbers are given for each continuous epitope and the mean displacement is calculated from $U^2 = 3B/8\pi^2$.

linear synthetic peptides corresponding to parts of proteins are able to elicit antibodies that react with the intact antigen^{28-30,33,55}. One explanation is that synthetic peptides are able to mimic the corresponding regions of the native protein because one of the conformations adopted in solution approximates that in the native molecule³³. In the so-called stochastic model, this native-like conformation of the peptide is assumed to be rare. An attempt to test this model involved estimating the frequency with which monoclonal antibodies directed against peptides recognize the corresponding intact protein⁵⁶; of 21 hybridomas that synthesized antibody against an influenza haemagglutinin peptide, 16 produced antibodies that reacted with the complete molecule. Such a high frequency seems to refute the view that the immunogenic form of the peptide corresponds to a rare conformational occurrence. However, the results described for haemagglutinin are rather unusual, as conventional antibodies raised against the complete molecule did not react with any of 20 peptides representing nearly the entire sequence of the molecule⁵⁵. Furthermore, it is also possible that the anti-peptide monoclonal antibodies recognized the intact haemagglutinin because this molecule had been partly denatured by the immunoassay procedures used in the experiments. It seems, therefore, that the stochastic model has not yet been adequately tested.

There is also an apparent contradiction between the finding that, on the one hand, monoclonal antibodies raised against a native protein are so conformation-specific that they usually do not bind to peptide fragments of the molecule, while, on the other hand, the majority of monoclonal antibodies raised against a peptide fragment also recognize the intact molecule^{48,54,56}. This contradiction might disappear if truly native, that is, correctly folded, intact, molecules were tested for their ability to react with anti-peptide monoclonal antibodies.

The obverse explanation offered for the finding that anti-peptide antibodies specific for different conformations of the peptide bind to the complete molecule is that local disorder in short segments of the protein mimics the flexibility of the peptide in solution⁵⁶. The data shown in Figs 2 and 4 represent the first concrete evidence of a link between high local mobility in proteins and the location of (continuous) antigenic determinants, but it should be stressed that this motion is generally one about a well-determined average conformation, even though the r.m.s. displacements in the cases of TMVP and lysozyme are of the order of 1–1.5 Å (Figs 2, 4b). The mobility in the epitope may make it easier to adjust to an antibody site which

after all has not been fashioned to fit the exact geometry of a particular protein. It may be necessary, therefore, to discard one of the assumptions of protein immunochemistry according to which epitopes in intact proteins are conformationally rigid while epitopes in short peptides are highly flexible.

The view that linear peptides in solution necessarily comprise a very large number of conformers, only some of which are recognized by protein-specific antibodies, arose from the finding that large molar excesses (of the order of 10^3 – 10^4) of peptide over intact protein are needed to reveal their antigenic reactivity^{4,5}. However, it is also possible that most antigenically active linear synthetic peptides contribute only a short stretch within them to a larger discontinuous antigenic region, and thus that the low level of antigenicity is not simply due to incorrect folding of the peptide.

Thus, the question remains of explaining the success that has been achieved in raising anti-peptide antibodies that are able to recognize apparently native proteins. It is conceivable that the extremes of segmental motion of proteins of the kind demonstrated here may provide the necessary degree of conformational similarity between the isolated peptide and the same region in the intact molecule. Epitopes located in flexible segments of the protein would be recognized in the form of peptides when the conformational prerequisites for building the antigenic structure are not very stringent—this is the case for the epitopes of TMVP and myoglobin that were identified by measuring the antigenicity (Fig. 2) and/or immunogenicity (Fig. 4a) of short peptides by using polyclonal antisera. In general, therefore, antibodies raised against a peptide would have a high probability of recognizing the corresponding epitope in the complete protein, whereas monoclonal antibodies raised against the intact protein would tend to be directed against the discontinuous epitopes which are more numerous^{51,52,54}.

Although segmental flexibility in proteins emphasizes only one type of antigenic determinant, its understanding is of crucial importance as it is probably on this type of epitope that present hopes for successful synthetic vaccines are ultimately based. Methods for predicting the secondary structure of proteins achieve a better success rate for loops and turns than for helices and sheets⁵⁷. As loops and turns are more likely to correspond to flexible segments of proteins, peptides corresponding to sequences predicted to lie in these positions should be good candidates for synthetic vaccines.

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Antibodies to gap-junctional protein selectively disrupt junctional communication in the early amphibian embryo

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*Antibodies to the major protein of rat liver gap junctions, molecular weight 27,000 (27K), have been microinjected into one identified cell of 8-cell stage *Xenopus* embryos. This treatment selectively disrupts both dye transfer and electrical coupling between the progeny cells. These results provide evidence that the 27K protein is an integral component of the cell-to-cell junctional channel. The disruption of junctional communication at early stages results in specific developmental defects, suggesting that blocking intercellular communication can have a pronounced influence on embryonic development.*

CELLS within the embryos of most species are able to communicate directly with each other through a low electrical resistance pathway. This pathway, which probably reflects the presence of the morphological structure, the gap junction¹, has been described in all embryos so far studied, both vertebrate and invertebrate². Communicating junctions are found between embryonic cells, regardless of their eventual developmental fate, at times when other evidence suggests that they are capable of influencing each other. This has led to the suggestion³ that the direct communication pathway mediated by gap junctions is responsible for the cell-to-cell transfer of information involved in directing embryonic development. Despite widespread support for this view, there is little evidence to indicate that gap junctions play a pivotal role in embryonic development.

There are a number of methods for reducing the permeability of gap junctions: raising intracellular calcium⁴, lowering intracellular pH (ref. 5) or treatment with alcohols⁶. However, all of these methods elicit secondary metabolic effects, making it difficult to relate unequivocally any effects these agents might have on development with their ability to lower gap-junctional permeability.

In recent years it has become possible to isolate and characterize constituent proteins from isolated gap junction preparations^{7,8}. This offers the opportunity to generate gap junction-specific antibodies which may be used to study the physiological properties of gap junctions. Here, we report the results of experiments to test whether antibodies raised against the major gap-junctional protein are able to interfere with cell-cell communication. In addition we have examined the developmental consequences of treatment with such antibodies.

Antibodies

For this analysis, three different affinity-purified rabbit polyclonal antibodies were selected. Polyclonal antibodies were preferred, as monoclonal antibodies are generally highly species-specific and rarely directed against functional portions of the molecule. Two of the antibodies (A and B) were generated by immunizing rabbits with a 27,000 molecular weight (27K) protein, the major protein present in rat liver gap junctions. The 27K protein was electrophoretically eluted from rat liver gap junctions which had been prepared as described in ref. 9. The sera from these two rabbits were purified on an affinity column containing only the eluted 27K protein. The resulting affinity-purified reagents bound specifically to the denatured 27K protein on immunoblots, as well as to 47K and 54K proteins present in homogenates of rat liver (Fig. 1a (1-3), b (1-3)). The 47K protein represents a 'dimeric' form of the 27K protein^{7,8}, while the relationship between the 54K and the 27K proteins in rat liver has not yet been clarified. All three of these molecules are recognized by the antibody that was affinity purified on the 27K column, as absorption with the 27K protein removes staining

of the 47K and 54K bands. In addition, the affinity-purified antibodies bind to the cytoplasmic surface of intact rat liver gap junctions as indicated by electron microscopic immunolocalization both on isolated gap junctions and in intact liver. A detailed description of the preparation and characterization of the antibodies will be published elsewhere (N.B.G. *et al.*, in preparation). Immunochemical evidence for the existence of related determinants in *Xenopus* embryos was obtained by applying antibody B to a nitrocellulose blot containing the 27K rat liver gap junction protein, a rat liver homogenate, and homogenates of various stage *Xenopus* embryos (see legend to Fig. 1). The immunoblot in Fig. 1 provides direct evidence that antigenically related proteins, comparable in size to those found in rat liver, are present in unfertilized *Xenopus* eggs and embryos. The 54K molecule, present in homogenates of both rat liver and *Xenopus* embryos, is the most abundant antigenic species that has been detected in immunoblots of *Xenopus* homogenates (unfertilized eggs, fertilized eggs, 8-cell, and 32-cell embryos). As noted earlier, the relationship between the 27K protein and the 54K antigenic species has yet to be clarified both in rat liver and in *Xenopus*. The 54K protein could represent a biosynthetic precursor of the gap junction, as indicated by Epstein *et al.*¹⁰ on the basis of the speed with which junctions can form between communication competent cells; and the ability to form junctions in the prolonged absence of protein synthesis and ATP production. Alternatively, the 54K protein may represent the undegraded gap-junctional protein as elimination of endogenous protease activity when preparing plasma membranes is very difficult. However, this uncertainty does not invalidate conclusions about the ability of the antibody raised against gap junctional protein to interfere with cell to cell communication, which are drawn from the results given below. The third antibody (C) was generated against, and specifically interacted with, an extracellular matrix glycoprotein that is synthesized and secreted by rat liver and *Xenopus* embryos. There was no cross-reactivity between this antibody and any of the species recognized by the antibody raised against gap junction protein, or with gap junctions. Conversely, the gap-junction antibody does not recognize the extracellular matrix glycoprotein. The 54K protein recognized by the gap junctional antibody cannot, therefore, be a contaminant from the extracellular matrix glycoprotein. Antibody C was therefore used as a control antibody that did not interact with gap junctions or the 27K gap-junctional protein.

The presence of gap-junctional proteins in the unfertilized egg may seem surprising. However, in the early *Xenopus* embryo, zygotic RNA and protein synthesis does not begin until the 'midblastula transition' (ref. 11). All material necessary for progression through development to the blastula stage must, therefore, be available in the unfertilized egg. This would include the production of gap junctions, as well as many other cellular

products. For example, it has been reported that fibronectin is present in early *Xenopus* embryos 2 h after fertilization and subsequently as a product of maternal transcripts¹², and large quantities of sodium are sequestered in the egg for subsequent transfer to the blastocoel fluid as the blastocoel cavity forms¹³.

To test whether these antibodies were capable of interfering with intercellular communication, they were injected into

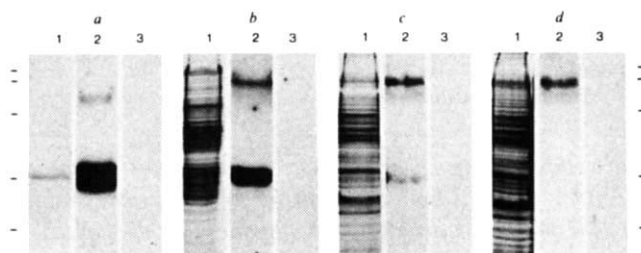


Fig. 1 Immunoblot analysis of rat liver gap-junction protein, rat liver homogenate, and *Xenopus* homogenates with pre-immune and immune affinity-purified antibody B. Lanes *a* (1–3) contained electrophoretically eluted rat liver 27K gap-junction protein, *b* (1–3) contained a protein homogenate from rat liver, *c* (1–3) contained a protein homogenate from *Xenopus* unfertilized eggs and *d* (1–3) contained a protein homogenate from 8-cell stage *Xenopus* embryos. The protein in lanes labelled 1 was separated electrophoretically and stained with Coomassie brilliant blue. Samples in all other lanes were transferred to nitrocellulose paper and treated with either the immune antibody B at 1:100 dilution (lanes 2), or with pre-immune antibody B at 1:100 dilution (lanes 3). The bound immunoglobulin was detected with an autoradiogram following treatment with ¹²⁵I-labelled *Staphylococcus* protein A. Molecular weight standards are indicated by lines to the left and right of the figure; they represent (from top to bottom) 68K (bovine serum albumin), 57.5K (catalase), 43K (ovalbumin), 25.7K (chymotrypsinogen), and 21.5K (soybean trypsin inhibitor). Note that the 27K junction protein was detected in both the Coomassie blue stained material (lane *a*, 1) and the autoradiograms of the immune antibody-treated material from the eluted 27K protein (*a*, 2), the liver homogenate (*b*, 2), and *Xenopus* unfertilized eggs (*c*, 2). The dimeric form of the 27K protein was readily detected in the eluted 27K preparation (*a*, 2), and minor amounts were detected in the other samples (*b*–*d*, 2). With the same reagent, a slightly larger molecule (about 54K) was detected in rat liver homogenates (lane *b*, 2). The 54K protein was also observed in *Xenopus*, and it was the most abundant antigenic species detected (lanes 2, *b*, *d*). No antigens were detected in any of the samples by the pre-immune reagents, lanes 3.

Methods: The *Xenopus* samples were prepared by collecting at appropriate stages (unfertilized eggs, fertilized eggs, 8-cell stage, and 32-cell stage), removing the vitelline membranes and jelly by dissection, and homogenizing material in 1 mM NaHCO₃ with a loose-fitting Dounce homogenizer. The homogenate was spun at 500g for 10 min in a Beckman TJ-6 centrifuge to remove most of the yolk and pigment granules. The remaining supernatant was spun at 12,900g for 15 min in an Eppendorf centrifuge. Both pellets and supernatants from these samples were analysed immunochemically and found to contain immunoreactive material. The samples in this figure represent the 12,900g supernatants from the specimens of unfertilized eggs and 8-cell stage embryos. The different protein samples were solubilized in a SDS sample buffer²² and electrophoretically separated with a 12.5% SDS-polyacrylamide slab gel. The gel was stained with Coomassie blue (2.5% Coomassie blue (w/v), 50% methanol, 7% glacial acetic acid) for about 15–20 min and then destained (40% methanol, 7% glacial acetic acid). For immunoblotting, the protein was electrophoretically transferred from the gel to nitrocellulose paper (BA85; Schleicher and Schuell) according to the published procedure²³. The paper was initially 'blocked' by treatment with 3% bovine serum albumin (BSA) in Tris buffered saline (TBS) overnight at 4 °C, and then the appropriate antibody was added at a 1:100 dilution in 3% BSA-TBS for 2 h at room temperature. Following several washes in TBS containing 0.1% Nonidet P-40 (NP40), the blot was incubated for 1 h at room temperature with ¹²⁵I-labelled *Staphylococcus* protein A (ICN) (35 Ci g⁻¹ protein). The blot was then washed with TBS-NP40, then TBS alone, dried, and placed on Kodak XAR film with an intensifying screen at -70 °C. The film was exposed for 3 days.

Xenopus embryos at the 8-cell stage. The injections were always made into the same cell, located in the region of the grey crescent (Fig. 2a). Assays of intercellular transfer were made two cleavage divisions later at the 32-cell stage (Fig. 2b), which allowed sufficient time for the antibody to diffuse throughout the four daughter cells. To confirm that the cells contained the injected antibody, a second reagent, fluorescein isothiocyanate, (FITC)-conjugated goat anti-rabbit IgG, was applied directly to the antibody-injected region on frozen sections. The localization of antibody at the edge of the injected region is illustrated in Fig. 3c.

Transfer of Lucifer yellow

The pattern of cell–cell transfer of Lucifer yellow in the 32-cell *Xenopus* embryo is described elsewhere in this issue¹⁴. In the present experiments, injection of Lucifer yellow were made into one of the cells showing widespread transfer of dye to its lateral neighbours, cell *d* (see Fig. 2b), which is one of the progeny of the antibody injected cell. Following injection of either gap-junctional antibody A or B, cell *d* rarely transferred dye to its neighbours. Figure 3b is a photograph of an intact embryo after injection of Lucifer yellow into cell *d* of an embryo previously injected with antibody B. The adjacent frozen section (Fig. 3a) confirms that the Lucifer yellow remains confined to the injected cell. For comparison, a frozen section taken through an embryo initially injected with pre-immune serum B is shown in Fig. 3d, demonstrating extensive transfer of Lucifer yellow to its lateral neighbours. The results of all assays of Lucifer yellow transfer in this series of experiments are shown in Table 1. None of the injected reagents influenced cell membrane potential or cell division. In each set of control embryos, failure to transfer to one or more cells, other than the sister of the previous cleavage, was only observed in about 30% of the embryos tested. In contrast, between 70 and 80% of the embryos injected with antibody A or B failed to transfer dye; transfer failed to take place into cells lying on both sides of the injected cell (see Fig. 3a). This implies that the antibodies can block, or reduce, the permeability both of junctions formed before antibody injection, that is, those present at the 8-cell stage, and possibly of junctions formed during the subsequent cleavage to 16 cells. It is also possible that the antibody can inhibit the formation of junctions in later cleavage stages.

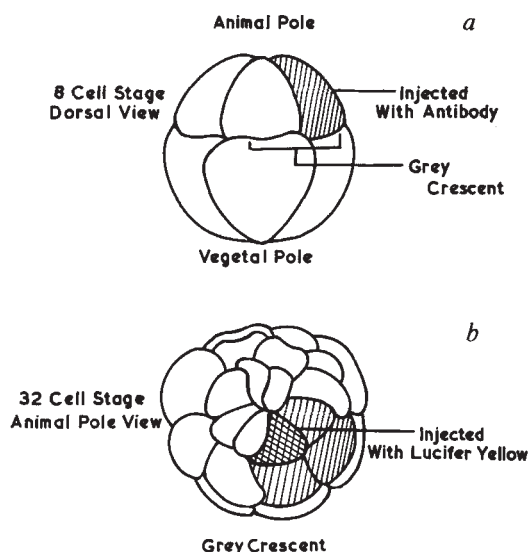


Fig. 2 The location of the cell injected with antibody at the 8-cell stage (*a*) and subsequently with Lucifer yellow, cell *d* (*b*). Hatched region indicates the presence of injected antibody. Cross-hatched region the presence of injected Lucifer yellow. ~15 nl antibody was pressure-injected using a Picospritzer. Lucifer yellow was injected ionophoretically at a current of 100 nA for 5 min with 30 s pulses at 10 Hz. Transfer of Lucifer yellow was assayed at the end of the injection period.

Table 1 Incidence of dye passage in 32-cell stage *Xenopus* embryos after injection of gap-junction antibodies

Condition	Transfer to more than one cell, excluding sister % (n)*	No transfer, or to sister cell only % (n)
Control 1	69 (11)	31 (5)
Control 2	63 (10)	38 (6)
Control 3	79 (11)	21 (3)
Pre-immune A	86 (12)	14 (2)
Pre-immune B	75 (12)	25 (4)
Immune A (I+II)	26 (13)	74 (37)†
Immune B (I+II)	18 (9)	82 (41)†
Immune C (I+II)	76 (40)	34 (21)

Control 1, values taken from ref. 14. Control 2, buffer containing 7.5 mM NaCl, 2.5 mM Na borate, 10 mM boric acid, pH 7.4. Control 3, buffer containing 5 mM Tris, 75 mM KCl, pH 7.6. Pre-immune A, IgG from rabbit A serum obtained prior to immunization injected at 1.5 mg ml⁻¹. Pre-immune B, IgG from rabbit B serum obtained prior to immunization injected at 1.5 mg ml⁻¹. Immune A, affinity-purified IgG from rabbit A after immunization with electrophoretically eluted 27K protein from rat liver gap junctions. Immune B, affinity-purified IgG from rabbit B after immunization with electrophoretically eluted 27K protein from rat liver gap junctions. Immune C, affinity-purified IgG from rabbit C after immunization with electrophoretically eluted glycoprotein from rat liver extracellular matrix. I, reagent affinity-purified X1, injected at a concentration of 1 mg ml⁻¹ in buffer 2 or 3. II, reagent affinity purified X2, injected at a concentration of 1.5 mg ml⁻¹ in buffer 2 or 3. Results with I and II were identical and were therefore combined in the table.

Methods: All injections were made at the 8-cell stage, in a volume of ~15 nl. Transfer of Lucifer yellow out of cell *d* (for nomenclature see ref. 14) was assessed at the 32-cell stage. Transfer to sister cell only was scored 'no transfer' because of cytoplasmic bridges remaining after cleavage. Transfer was assessed immediately after injection of the dye (5–7 min of injection). The affinity purified reagents were prepared chromatographically²¹ using either the 27K protein (antibodies A and B) or the glycoprotein (antibody C) conjugated to CNBr-activated Sepharose 4B (Pharmacia). The sera were applied to the columns and the bound IgG reagents were eluted by treatment with 200 mM glycine, pH 2.6. The reagents were then dialysed against either 0.1 × control buffer 2 or 3, and concentrated 10-fold with a Savant Speed-Vac before use. IgG was obtained from the pre-immune sera by using a Protein A-Sepharose 4B (Pharmacia) column; the bound IgG was eluted by treatment with 100 mM glycine, pH 3.0.

* *n* = no. of embryos tested.

† Incidence of transfer significantly different from controls (χ^2 test, $P < 0.005$).

To confirm that the antibody was able to block junctions that were already formed, antibody B was injected into cell *d* at the 32-cell stage; this was immediately followed by injection of Lucifer yellow. Subsequent assay for dye transfer took place within 5 min of antibody injection (the minimum time for ionophoretic injection of Lucifer yellow). Of 12 embryos tested, 10 failed to transfer dye. This is significantly less than in control embryos ($0.01 > P > 0.005$). We therefore conclude that the antibody blocks transfer through intact junctions within a short time. It is unlikely that the block is the result of an increase in intracellular calcium, since direct injection of calcium ions will not block gap junctions in *Xenopus*¹⁵. A fall in intracellular pH (pH_i) is also unlikely, as cell division is inhibited when pH_i falls sufficiently to block cell–cell communication⁵; cleavage was not affected by injection of antibody. The question of whether the antibody also inhibits the formation of new gap junctions in subsequent developmental stages is more difficult to resolve. This issue must await further experiments designed to analyse the structure of gap junctions in antibody-injected cells several cleavage stages later.

To confirm that these effects were the consequence of the presence of gap-junctional antibody, a number of other controls were carried out. Injection of an antibody to an extracellular matrix glycoprotein (antibody C) had no effect on the transfer pattern (Table 1). Injection of pre-immune sera from the rabbits

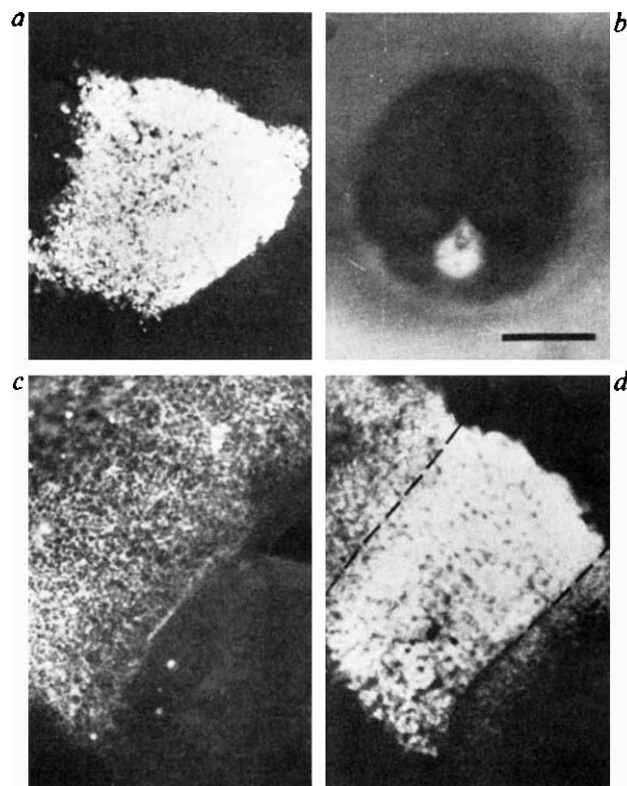


Fig. 3 *a*, Frozen section taken at the 32-cell stage through cell *d* to show restriction of Lucifer yellow to the injected cell after prior injection of antibody B at the 8-cell stage. *b*, Whole embryo injected with antibody B at the 8-cell stage, showing restriction of Lucifer yellow subsequently injected at the 32-cell stage. *c*, Frozen section at the 32-cell stage through embryo injected with antibody B at the 8-cell stage showing antibody stained with FITC-labelled goat anti-rabbit antibody (Nordic Laboratories) applied directly to the frozen section. Note clear restriction of antibody to one of the progeny of the cell injected at the 8-cell stage. *d*, Frozen section through cell injected with Lucifer yellow at the 32-cell stage after injection of pre-immune serum B at the 8-cell stage. Note extensive transfer to both lateral neighbours. Cell borders are indicated by dotted lines. Compare with *a*. Scale bar, 150 μ m for *a*; 500 μ m for *b*; 100 μ m for *c* and 250 μ m for *d*. For frozen sectioning, embryos were briefly fixed (1 h) in 4% formaldehyde, frozen in isopentane and 20 μ m serial sections were taken on a Bright 5030 cryostat.

used to raise antibodies A and B also did not interfere with the pattern of transfer, which was similar to that observed in control embryos (Table 1). Thus only injection of antibodies raised against gap-junctional protein interfered with dye transfer.

Similar experiments were carried out with monovalent fragments of the affinity-purified immunoglobulins, prepared from immune antibody B (refs 16, 17), in order to determine whether the disruption of communication was due to cross-linking of the multivalent IgG on the cytoplasmic surface of the junction, or to a direct interaction of the immunoglobulin with the 27K protein in the channel region. With non-immune Fab fragments (Cappel Laboratories; injected at 1.5 mg ml⁻¹) dye transfer failed in only 20% of the embryos, whereas with the immune fragments (injected at 1.5 mg ml⁻¹) dye transfer failed in 52% of the embryos tested. The incidence of failure to transfer observed after injection of the immune fragments is significantly greater than that observed with the non-immune fragments ($0.1 > P > 0.05$), suggesting that disruption of the channel permeability results from a direct interaction of the antibody with the 27K protein.

Effects on electrical coupling

Dye transfer provides a relatively insensitive measure of cell–cell communication, as low levels of transferred dye may not reach the threshold for detection. To confirm that failure of dye

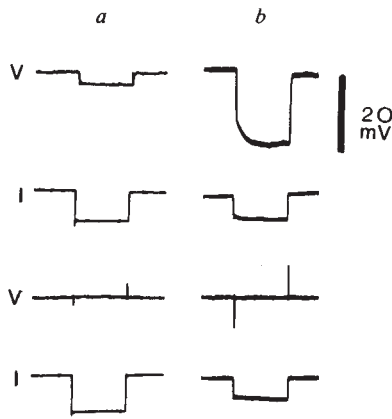


Fig. 4 Electrical coupling between cells injected with either antibody A (a) or antibody B (b). Embryos were released from the vitelline membrane and the seal between the blastocoel and extracellular fluid mechanically broken to prevent current flow through the extracellular space. One electrode injected hyperpolarizing current pulses (1 s long, 1×10^{-8} A in a and 3×10^{-8} A in b; current was recorded at different gains in a and b) into one cell while a second electrode inserted into a lateral neighbour recorded the resultant voltage deflection. Current and voltage recorded using conventional electrophysiological techniques and displayed on an oscilloscope and Gould-Brush pen recorder. Upper pairs of traces show electrical coupling between two cells in the uninjected region. Lower pairs show the absence of electrical coupling in the antibody containing region of each embryo; in both cases only capacitive artefact remains on the voltage trace. Membrane potential was -40 mV (a) or -30 mV (b). Membrane potentials in the injected and uninjected regions were within 5 mV of each other.

transfer was accompanied by a reduction in electrical coupling, embryos which had been injected with Lucifer yellow and scored for failure to transfer dye were tested for the presence or absence of electrical coupling. In the whole embryo current can flow from one cell to the next through the restricted extracellular space because of the presence of tight junctions joining the outer edges of the cells. To avoid this complication, the embryos were released from their vitelline membranes and the seal into the blastocoel was broken mechanically before testing for electrical coupling. In each embryo, coupling was tested between cells lying in the uninjected animal pole region of the embryo for comparison with coupling between cells containing either antibody A or B. Figure 4 shows the results for two embryos. Injection with either antibody A (Fig. 4a) or antibody B (Fig. 4b) reduced electrical coupling to below the limits of detection. Similar measurements on 10 other embryos revealed either complete abolition of electrical coupling (8 cases), or a reduction to just above the detectable level (2 cases), between cells containing either gap-junction antibody.

The occasional failure of the antibodies to cause complete block of both dye transfer and electrical coupling is almost certainly the consequence of a large, potentially variable, amount of unassembled junction protein available in the egg. Undoubtedly, variability in the amount of antibody injected also contributes. Presumably all of the protein available to form junctional channels must combine with the antibody in order for the block to be completely effective.

These results show that two antibodies raised against a protein extracted from rat liver gap junctions are capable of blocking functional transfer of both a low molecular weight dye and small ions in the early *Xenopus* embryo. Since preliminary experiments to test the efficacy of such antibodies in a cultured rat liver cell line showed that antibody A prevented cell to cell transfer of 6-carboxyfluorescein, the results suggest that gap junctions in both *Xenopus* and rats share a determinant which is closely involved in controlling the transfer of molecules from cell to cell. This is consistent with the observed immunochemical homology (Fig. 1) and a previous report on the lack of specificity

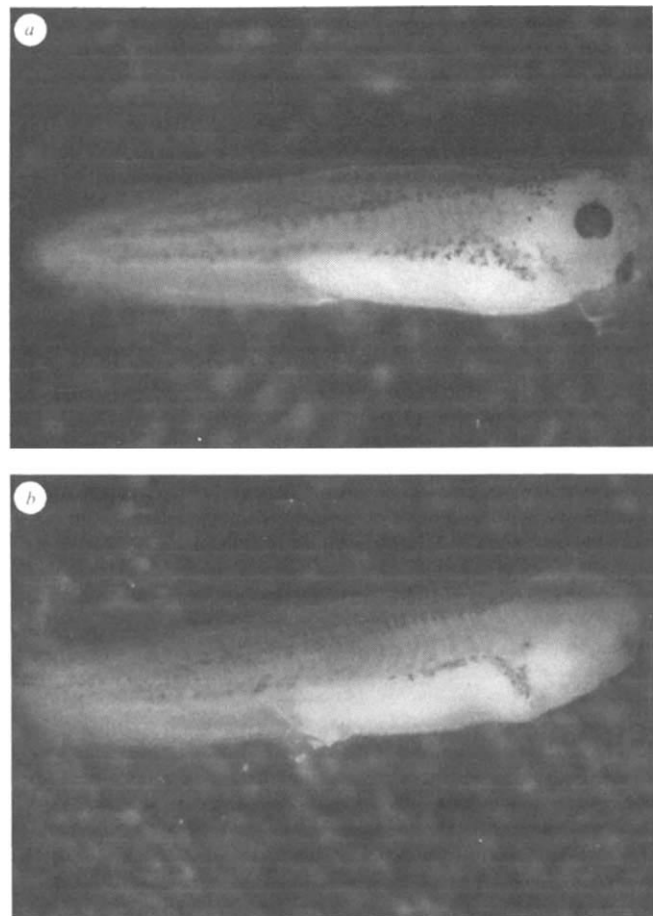


Fig. 5 A control embryo (a) and an embryo injected with antibody A at the 8-cell stage (b) photographed by S. E. Fraser prior to fixation, when the control had reached stage 36. The embryos are viewed from the right hand side. Note absence of the eye and deformed shape of the head in the antibody injected embryo. Note that the tail of the injected embryo is fairly normal. Sections through these two embryos to show the internal head structures are shown in Fig. 6.

for generating cell to cell channels between heterologous vertebrate cells¹⁸.

Developmental consequences

Cells injected with all the reagents tested, maintained normal resting potentials through many rounds of cell division and continued to divide at the same rate as other cells in the embryo. The injected embryos were examined for signs of cell death and extrusion throughout their development but no signs of death were observed. Embryos in which the injected cell was killed or injured (by deliberate over-irradiation after injection of Lucifer yellow) showed gradual failure of cleavage and cytolysis of the progeny of the injured cell. Such effects were never seen in embryos injected with gap junction antibody. Furthermore, the progeny of the cell which had been injected with Lucifer yellow at the 32-cell stage to test for dye transfer after antibody injection, were clearly visible in the living tadpole for at least 3 days. The progeny of the cell injected at the 8-cell stage contribute to both ectodermal and mesodermal derivatives on the right hand side of the embryo. Injection of either antibody A or B generated a high proportion of tadpoles with a characteristic set of abnormalities which were related to the developmental fate of the injected cell. The most frequently occurring defects were varying degrees of right/left asymmetry (63%), although some embryos (19%) failed to form brains and eyes on both sides. In asymmetric embryos the right eye frequently failed to form altogether; this was accompanied by gross underdevelopment of the brain on the right hand side. An example

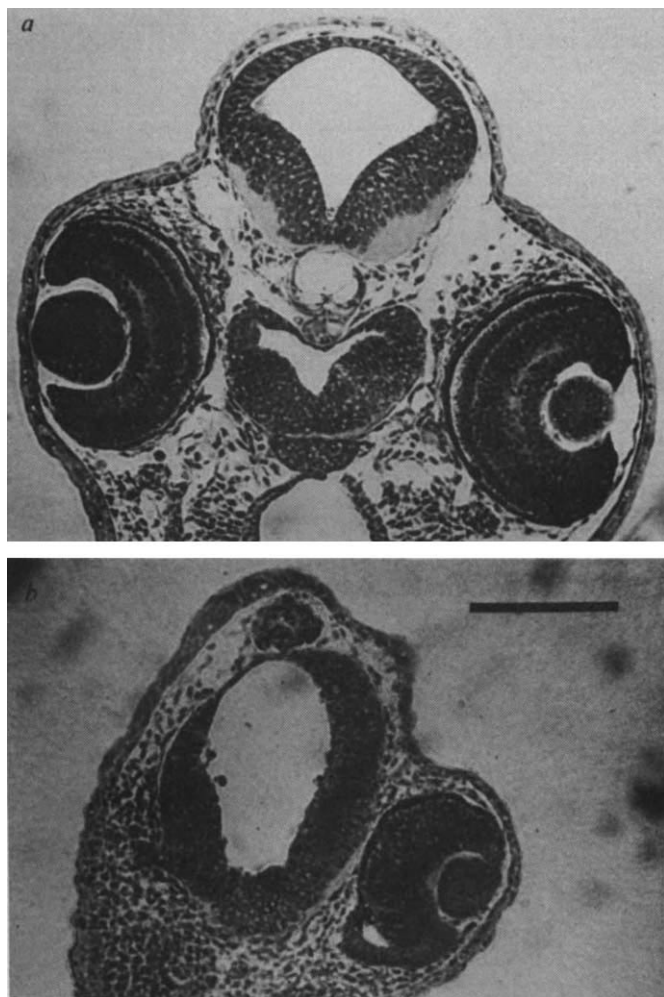


Fig. 6 Transverse section at the level of the developing eyes in a control embryo at Nieuwkoop and Faber stage 36 (ref. 24) (a) and an embryo of the same age which had been injected with antibody A at the 8-cell stage (b). Scale bar, 500 μ m. The plane of section through the two eyes of the control embryo is not exactly the same because of a slight tilt in orientation. The experimental embryo shows three features of note: (1) the eye is missing on the side previously injected with gap-junction antibody; (2) the brain is the shape normally found anterior to the eyes and (3) the brain is particularly underdeveloped on the injected side. Embryos fixed in 2.5% glutaraldehyde in Ringer's solution overnight, washed in buffer, dehydrated in alcohols and embedded in methacrylate resin. 7 μ m serial sections were cut on a Sorvall JB4 microtome and stained with haematoxylin and eosin.

of this defect in the intact embryo, produced by injection of antibody A, is shown in Fig. 5, along with a photograph of its sibling control (Fig. 5a). Figure 6 shows sections taken at the level of the eyes through these two embryos. In milder cases of impaired development, the notochord was shifted from its central position to the uninjected side and the brain and eyes on the right hand side were then much smaller than those on the left. Attempts to produce completely communication-incompetent embryos, by injecting antibody just before the first

cleavage, or into both cells at the two cell stage, were unsuccessful; all such embryos died before gastrulation. None of the control reagents induced abnormalities above the spontaneous, low level. The abnormalities observed in control embryos were highly variable and rarely asymmetric.

Until we know how long the injected gap-junction antibody remains effective in blocking cell to cell communication and how the embryo responds to the presence of a group of non-communicating cells, it is difficult to relate the observed developmental defects directly to a role for gap-junctional communication. Nevertheless, the developmental defects induced by injection of antibodies to gap-junctional protein clearly warrant careful and detailed analysis.

Our studies show that two antibodies raised against the 27K gap-junction protein extracted from rat liver, are able to inhibit functional cell to cell communication in the early *Xenopus* embryo, assessed either as transfer of the dye Lucifer yellow or by electrical coupling. These observations provide direct evidence that the 27K protein in gap junctions is an integral component of the functional channel for cell-cell communication. Several studies have suggested that the channel is formed by the oligomeric arrangement of proteins^{19,20}; this suggestion is clearly consistent with our findings. Furthermore, the disruption of intercellular communication caused by injection of antibodies to the 27K protein produces characteristic developmental defects. It should therefore now be possible: (1) to identify a molecular determinant(s) which controls the functional properties of gap junctions; (2) to further analyse the developmental consequences of blocking cell to cell transfer, in order to determine the developmental role of intercellular communication.

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Detection of molecular hydrogen in two merging galaxies

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NGC6240 and Arp 220 (IC4553) are two of the most luminous IR galaxies known^{1,2}. They are also both thought to be examples of galaxy-galaxy mergers. As part of our study of interacting and merging galaxies^{1,3,4} we have obtained IR spectra in the 2- μ m region of NGC6240 and Arp 220 and detected the $\nu = 1-0$ S(1) quadrupole rotation-vibration emission line of H_2 (rest wavelength 2.122 μ m) in both galaxies. (This line in NGC6240 has also recently been detected by E. E. Becklin *et al.*, personal communication.) These detections, which double the number of previously published measurements of extragalactic H_2 (refs 5, 6) suggest that the merger of two galaxies results in the production of massive quantities of shocked molecular gas. This shocked gas must cool and collapse, leading to an enormous burst of star formation. These measurements of shocked H_2 thus strengthen our earlier interpretation¹ of the extremely large IR luminosity of NGC6240 in terms of a 'super-starburst'. Arp 220 may be undergoing similar activity. We suggest that merging galaxies are a unique new class of ultra-luminous IR galaxies.

The spectra were measured on the UK Infrared Telescope (UKIRT) on the nights of 10 and 11 April 1984 using a seven-channel cooled grating spectrometer (CGS-2) with a 5 arc s aperture⁷. Wavelength calibration and the instrumental resolution of 550 km s⁻¹ were derived from observations of Brackett γ (λ 2.166 μ m) in the planetary nebula NGC7027. Spectra of the star BS5447 provided flux calibration. The spectra were measured at positions defined by the peak of the IR emission for NGC6240, and by the peak of the optical emission for Arp 220. The spectral coverage of the seven-channel spectrometer, ~ 0.023 μ m, was centred on the $\nu = 1-0$ S(1) line of H_2 (hereafter, the S(1) line) redshifted to the recessional velocities of the galaxies. The full spectra obtained are shown in Fig. 1. The S(1) line is clearly detected in both galaxies and probably also resolved in both. The corresponding fluxes and luminosities, for $H_0 = 50$ km s⁻¹ Mpc⁻¹, are given in Table 1. The luminosity in the S(1) line for NGC6240 of $10^8 L_\odot$ may be compared with luminosities of $3 \times 10^6 L_\odot$ and $3 \times 10^7 L_\odot$ in this line for NGC1068 (ref. 5) and NGC3690 (ref. 6) respectively.

Using the data in Table 1 we can develop the following physical picture of the H_2 excitation in these two merging galaxies. First, this gas is almost certainly excited by shock-heating, as is the case for virtually all known examples of H_2 quadrupole emission⁸. In NGC6240, the width of the S(1) line, ~ 800 km s⁻¹, is identical to the widths of optical emission lines seen in a similar aperture on the nucleus, and which are thought to be shock-excited⁹. Fluorescence following absorption of UV photons is considered to be the most plausible alternative mechanism for excitation of these rotation-vibration levels. In this case, the $\nu = 2-1$ S(1) line should have about half the intensity of the $\nu = 1-0$ S(1) line¹⁰. Our failure to detect the $\nu = 2-1$ S(1) line in NGC6240 at a sensitivity similar to that reached for the $\nu = 1-0$ S(1) line detection, suggests that UV pumping does not contribute significantly to the excitation, and shocks remain as the most likely excitation mechanism.

Second, the mass of hot gas may be estimated by assuming local thermodynamic equilibrium at a temperature of 2,000 K (ref. 8). The luminosities in Table 1 then imply masses of excited

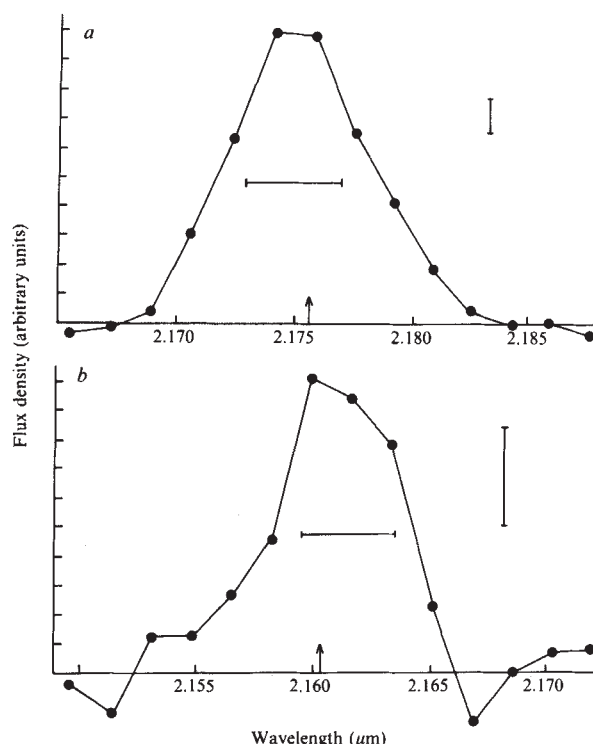


Fig. 1 Near IR spectrum of the nucleus of a, NGC6240; b, Arp 220 in a 5 arc s aperture. The arrow shows the wavelength of the 2.122 μ m S(1) line redshifted to the radial velocity of the respective galaxies. The spectrum has been ratioed with the standard, smoothed using a triangular function, and the baseline has been subtracted off. The vertical bar represents the 1σ error per point, and the horizontal bar represents the instrumental resolution.

molecular gas of $\sim 10^5 M_\odot$ and $\sim 10^4 M_\odot$ for NGC6240 and Arp 220 respectively.

Third, we can use simple energetic arguments to investigate the underlying source driving the shocks. The gas cools to a temperature $< 1,000$ K in about 3 yr (ref. 11), so in NGC6240 the shock excites about $3 \times 10^4 M_\odot$ of gas per year. The force, $\dot{m}v$, required to drive such a shock at a speed of ~ 20 km s⁻¹ is $\sim 5 \times 10^{36}$ dynes. By comparison, the radiation pressure, L/c , of these ultra-luminous galaxies, with $L_{IR} \sim 10^{12} L_\odot$, is $\sim 10^{35}$ dynes. Despite the high luminosities, radiation pressure is clearly insufficient to drive the shocks which we infer are present in these galaxies.

These objects are the results of galaxy-galaxy mergers, and are both likely to be examples of 'super-starbursts'^{1,2}. Two obvious mechanisms for driving the shocks are either the relative kinetic energy of the collision, or mass outflow from the early-type stars in the starbursts. To investigate the relative contributions of these two processes we can use the ratios of the luminosities in the S(1) lines to the total IR luminosities. This ratio should have a characteristic value if the H_2 is excited by mass outflow from young stars, and the IR luminosity is provided by the luminosity of the early-type stars which is thermalized by dust and re-radiated in the IR. These ratios are presented in Table 2, along with that for Orion, a 'typical' star formation

Table 1 Fluxes and luminosities in the S(1) line

Galaxy	Flux (W m ⁻²)	$L(L_\odot)$
NGC6240	$(15 \pm 1) \times 10^{-17}$	1×10^8
Arp 220	$(2.5 \pm 0.5) \times 10^{-17}$	9×10^6

Table 2 The ratio of luminosity in the S(1) line to total, IR luminosity

Object	Ratio ($L(S(1))/L_{IR}$)
NGC6240	20×10^{-5}
Arp 220*	1.2×10^{-5}
Orion	1.2×10^{-5}
NGC3690	3×10^{-5}
NGC1068	0.9×10^{-5}

* See comments in text.

region in the Galaxy¹². For comparison, we have also included these ratios for the other two galaxies in which H₂ has been detected, NGC1068 and NGC3690 (refs 6 and 12). We have used our 10–20 μ m UKIRT photometry¹ to obtain the appropriate IR luminosity for the nucleus of NGC6240 in a 5 arc s aperture, similar to that used for the S(1) observations. These measurements give an IR continuum spectrum of similar shape to the large aperture IRAS photometry¹ and imply a luminosity $L_{IR} \sim 5 \times 10^{11} L_{\odot}$, a factor of 4 less than that inferred from the IRAS results. We have, therefore, used a similar factor to scale the IRAS luminosity for Arp 220 (ref. 2) to that expected in a 5 arc s aperture.

Table 2 shows that all the galaxies, with the exception of NGC6240, could be excited by mass outflow from star formation regions similar to Orion. NGC6240 is clearly the exception, by a factor of more than 10, and it is likely that mechanical energy in the galaxy–galaxy collision has provided most of the energy required to heat the H₂ gas. If we assume a galaxy mass of $\sim 10^{12} M_{\odot}$, 10% of which is gas, and a relative velocity between the colliding galaxies of $\sim 300 \text{ km s}^{-1}$, then this process supplies an energy of $\sim 10^{59}$ ergs. For an efficiency of 0.002 in coupling shock energy into the S(1) line¹³, the mechanical energy in the collision can excite the gas at the observed rate for a time $\sim 2 \times 10^7$ yr. For Arp 220 the luminosity ratio in Table 2 is entirely consistent with excitation by mass outflow from star formation regions. However, it is very likely that we have not measured the maximum S(1) line intensity in Arp 220, since we observed only the optical nucleus. Given the physical similarity between NGC6240 and Arp 220, it would be surprising if a better measurement of the S(1) line flux did not result in a luminosity ratio closer to that for NGC6240.

There are several important conclusions to be drawn from these measurements. First, the existence of massive quantities of shocked molecular gas, in excess of that expected for star formation regions alone, is consistent with what might be expected when galaxies merge, and tends further to confirm other indications that NGC6240 is indeed the merger of two gas-rich galaxies. Second, both these galaxies are probably undergoing an episode of star formation of exceptional intensity, with shocked, and, therefore, dense H₂ produced at the rate of 3×10^3 to $3 \times 10^4 M_{\odot} \text{ yr}^{-1}$. This gas will cool rapidly, collapse and fragment, and result in a burst of star formation. The enormous mass of molecular gas that is shocked over any reasonable lifetime for this process, and its expected spatial extent on a scale of kpc if the shocks are merger-driven, as seems to be the case for NGC6240 at least, will produce an episode of rapid star formation of unusual spatial extent and intensity, that is, a 'super-starburst'. Finally, because dust is required to form the H₂ molecules¹⁴, there must be dust present. It will thermalize the radiation from the newly-formed stars and re-radiate it in the IR. Thus these super-starbursts are likely to produce ultra-luminous IR galaxies. We have suggested previously¹ that NGC6240 is a paradigmatic example of such a process, and the detection of shocked H₂ strongly supports this interpretation. As a consequence, merging galaxies may be a unique new class of galaxies exhibiting super-starbursts and ultra-large IR luminosities.

This picture of interaction-induced shocks, which then trigger a super-starburst in merging galaxies, is physically coherent and plausible. However, there remain some fundamental unan-

swered questions concerning the details of these processes. First, how common might such a phase of vigorous H₂ excitation be among merging galaxies generally? As these two galaxies were selected for study on the basis of their large IR luminosities, it is difficult to attempt generalizations about the time scale of the phase of luminous H₂ emission on the basis of these measurements alone. However, the fact that the S(1) line is so luminous in two merging galaxies suggests that the episode of this activity may be a significantly large fraction of the time over which the morphological disturbance would be recognized as a merger. The dynamical simulations of Toomre and Toomre¹⁵ and Wright¹⁶ suggest that the tidal 'tails' resulting from an interaction persist for periods of the order of 10^9 yr. This is longer than the 2×10^7 yr estimated above for the time merger-driven shocks can excite the gas in NGC6240 at the observed rate. Despite the rather large uncertainties in this estimate, the duration of luminous H₂ emission powered by the merger is unlikely to be more than 10^8 yr. Even if the H₂ were mostly excited by mass outflows from star formation regions, the time scale for such starbursts is probably $\leq 10^8$ yr (ref. 17). This is also rather short compared with the merger lifetime. So, if these time scales are approximately correct, we would expect that only $\sim 10\%$ of the galaxies recognizable as mergers from their morphologies are currently undergoing a phase of activity similar to that in NGC6240.

Perhaps even more intriguing is the fact that we failed to detect the Paschen α hydrogen recombination line in NGC6240 at a comparable sensitivity to that reached in the S(1) line measurements. (We measured Paschen α because Brackett α , at a rest wavelength of $4.051 \mu\text{m}$, is at the edge of the atmospheric transmission window at the redshift of NGC6240.) For those objects in which the H₂ excitation is thought to be due to mass outflows in star formation regions—NGC1068, NGC3690, and Orion—the Brackett γ line flux is within a factor of 2 of the S(1) line flux. Although we have shown that in NGC6240 the S(1) line is ~ 20 times stronger than expected for excitation from star formation regions, the Paschen α line is also expected to be about 12 times stronger than Brackett γ from recombination theory^{18,19}. Because it is difficult to avoid the conclusion that the high IR luminosity of NGC6240 is due to a massive burst of star formation¹, our failure to detect the Paschen α recombination line is indeed surprising. Whether this can be understood in terms of a complex geometry for dust extinction, self-shielding by the H₂, or perhaps a starburst initial mass function with fewer high mass stars, requires further detailed analysis. Such an analysis, supplemented by additional spectroscopic results for these and other interacting galaxies, is in preparation.

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Note added in proof: Molecular hydrogen emission has been discovered in these two galaxies by several independent observers as listed in IAU Circ. No. 3968.

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Detection of radiation from a heated and modulated equatorial electrojet current system

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In May 1983, ionospheric heating experiments were conducted using the Jicamarca very high frequency (VHF) radar facility located ~11 km east of Lima, Peru. Experiments involving high frequency (HF) heating of the lower D-region of the ionosphere were successfully conducted during 1982 using the Arecibo, Puerto Rico HF transmitter located 5 km east at Islote, Puerto Rico. These local experiments had characterized the signal radiated from a heated and modulated ionospheric current system near the mid-latitudes. A long-path signal had also been received in September 1982 at Salinas, Puerto Rico from a mid-day equatorial electrojet, heated and modulated by the Jicamarca facility. We have investigated the characteristics of the local signal that would be radiated from a strong equatorial electrojet when heated and modulated, and report here that at the geomagnetic equator they were similar to, but less intense than, those observed at Arecibo, Puerto Rico due to parameter differences. This radiation is believed to be the first detected from a heated and modulated equatorial electrojet current system in the Western Hemisphere.

The VHF heating experiments involved a research team from The Pennsylvania State University working in close cooperation with the Jicamarca Radar Observatory of the Geophysical Institute of Peru. Stubbe *et al.*¹ successfully detected radiation from the polar electrojet above Tromsø, Norway and Ferraro *et al.*² have detected radiation from the dynamo current system above Arecibo, Puerto Rico. The Peruvian experiment involved heating the equatorial electrojet current system with the Jicamarca radar (76°52' W, 11°57' S, dip 2° N). ~5,547 MW of effective radiated power (with linear polarization at 49.920 MHz and a 2° beamwidth, modulated at a 5% duty cycle with a square wave at a frequency of 2.5 KHz) was radiated vertically into the atmosphere. The radar was then operated on a heating and cooling cycle of 2 min on and 2 min off. This type of experiment has been repeated many times using the Islote, Puerto Rico heating transmitter of the Arecibo Observatory; however, the parameters of the Islote transmitter differ from those of Jicamarca. Specifically, the usual Islote heating frequency is 3.170 MHz and the duty cycle of the modulating signal is 50%. The two transmitting facilities are compared in Table 1.

The basic physics of electromagnetic radiation from a heated, modulated ionosphere at frequencies <30 KHz is influenced by the fact that ionospheric conductivity depends on the temperature of the electrons. The conductivity of the heated ionosphere varies periodically with the frequency of the heating radiation. The natural currents (such as, dynamo, polar electrojet, and equatorial electrojet) which pass through the heated region are modulated by conductivity changes, with radiation of energy at the modulating frequency having been detected.

The receiving system used a synchronous detector to measure the in-phase and quadrature components of the signal. A balanced loop 1 m in diameter consisting of 200 turns of number 14 wire with a tuned Q of ~55 at 2.5 KHz was used. A 0.5-m one-turn coaxially mounted loop was used for calibration using the procedure outlined in ref. 3. The signal was amplified, filtered, sampled and stored using a microcomputer with floppy diskette storage. Subsequent signal processing on the VAX 11/780 revealed the signal amplitude and phase as well

Table 1 Comparison of transmitting facilities

	Islote, Puerto Rico	*Jicamarca, Peru
Geographical location	18°28'33" N 66°39'57" W	11°57' S 76°52' W
Magnetic lat.	32° N	0°
Magnetic dip	51° N	2° N
Frequency (MHz)	3–12	49.920
Antenna	4 × 8 array of orthogonal planer log-periodic	96 × 96 array of orthogonal half wave dipoles
Antenna zenith gain (D)	23–26	42
Antenna beamwidth (deg)	5–10	2
Duty cycle (%)	Variable	5
Power (maximum) (kW)	800	350
Effective radiated power (MW)	160–320	5,547

* Values present during experiment.

as the Fast Fourier Transform (FFT) of the 2 min on–2 min off signal envelope.

The long-path experiment was conducted on 17 September, 1982 with the Jicamarca radar operating as previously described. The receiving equipment was located at Salinas, Puerto Rico (17°59' N, 66°16'45" W). Salinas is located ~67 km south east of the Islote transmitter and ~3,532 km from the Jicamarca Peru facility.

Figure 1 shows the 2.5 KHz FFT received on 17 September 1982 at Salinas, Puerto Rico. The dominant amplitude of 4.17 mHz corresponds to the on/off period of 240 s. The conversion of the amplitude to magnetic field strength at the loop was not accomplished for these data; however, when compared with the local primary component of 4.17 mHz, in volts, the local signal is stronger.

In May 1983 the receiving equipment was installed ~9 km south of the Jicamarca Observatory as shown in Fig. 2. A mountain range of several thousand feet separated the transmitter and the receiver. The transmitter was operated as previously described with two of four transmitters feeding the entire array. This limited the effective radiated power to 5,547 MW throughout the experiment. The high-power radiation capabilities were not utilized as the transmitter was undergoing modification at the time of the experiment. The radiation was linearly polarized with the electrical field in the north–south direction. The electrojet echo strength was monitored during the experiment with maximum returns near local noon. An antenna trap at 49.920 MHz was used to ensure that direct ground radiation would not affect the data. A VHF receiver was used to check the tone of the received signal from the transmitter. The tone scintillation on the 50 MHz receiver verified that there was a strong electrojet current during the received signal period.

The VHF heating test was conducted on 4, 5 and 9 May 1983. The dominant modulating frequency used was 2.5 KHz. Four hours of testing at 2.5 KHz during the first day were consumed in technical considerations of tuning receiving system calibration and general coordination to ensure valid test results. The receiving loop has its strongest quality factor at 2.5 KHz and a solid data base at this frequency from Arecibo testing was available for comparison. Only 1 h of testing was devoted to 5.0 KHz, 1.0 KHz and 0.08 KHz to complete a limited overall test schedule. No signals were found at these three frequencies; however, limited testing coupled with system parameters, past experience, and the need for tuning and calibration makes premature conclusions unwise.

The results at 2.5 KHz on 5 and 9 May 1983 were similar so only the latter are shown in Fig. 3. The signal radiated from the heated and modulated electrojet was observed within a few-hours window at local noon, chosen to coincide with a strong electrojet current. The received signal exhibited a cyclic character where it was visible in some portions of data and submerged in noise during others.

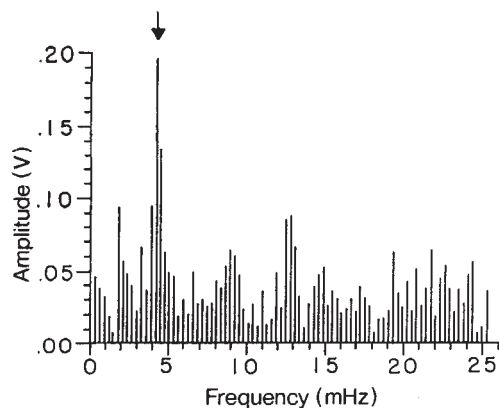


Fig. 1 Long-path amplitude spectrum, 2,500 Hz, Jicamarca, Peru to Salinas, Puerto Rico, 1600–1700 UT, September 1982.



Fig. 2 Relative locations of the Radar Observatory of Jicamarca, Peru and the VLF/ELF receiver location.

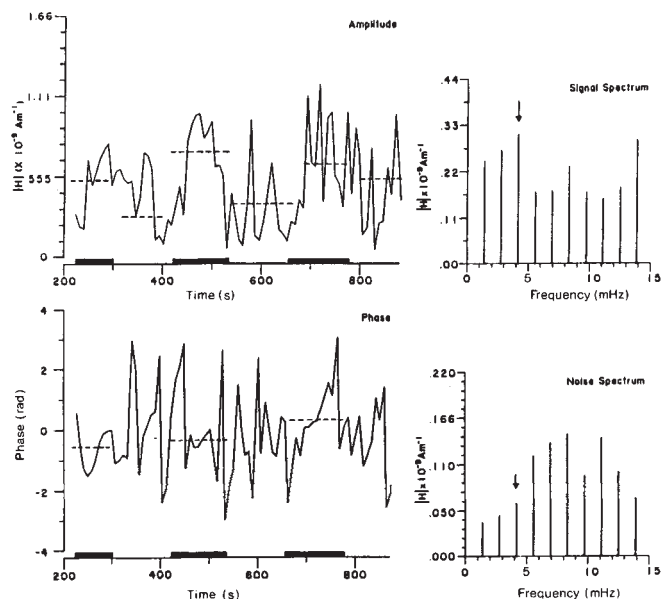


Fig. 3 Amplitude, phase and spectrum of a local Jicamarca, Peru signal at 2,500 Hz, 1345–1400 UT, 9 May 1983, compared with noise spectrum.

In Fig. 3, the amplitude and phase of the 2.5 KHz on 9 May are augmented with dashed lines to show average values. The intensity of the amplitude during the 2-min heating cycle, indicated by the dark portion of the time axis, was an order of magnitude less than a typical signal recorded at Arecibo. This is primarily attributed to the narrow beamwidth, higher heating frequency, and lower duty cycle of the Jicamarca radar compared with the Isote facility where parameters are closer to ideal. The phase tracking while the signal was being received is also characteristic of the Arecibo signal received. The amplitude spectrum shows the predominance of the 4.17-mHz spectral line. A pure noise spectrum is shown for comparison. The noise sample was recorded the day before testing because it could not be done while the experiment was in progress. This explains the apparent difference in amplitude of anything other than the primary frequency component. In this case, the calibration procedure was used to calculate the magnetic field strength at the loop, for comparison with Arecibo data recorded in 1983 and 1984.

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Bismuth in interplanetary dust

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Samples of a large ($\sim 60 \mu\text{m}$) chondritic porous (CP) aggregate collected from the stratosphere have been analysed in detail by analytical electron microscopy (AEM). Previous studies of CP aggregates have shown that they are extraterrestrial in origin^{1–3} and may be related to cometary debris⁴. CP aggregates are dissimilar to C1 and C2 carbonaceous chondrite matrices and many have not been significantly altered by thermal or radiation processes since their assembly⁵. We report here a high concentration of Bi_2O_3 grains within the large CP aggregate designated W7029*A ($\sim 60 \mu\text{m}$) and suggest they formed by rapid heating ($\sim 300^\circ\text{C}$) of elemental Bi grains within the aggregate during atmospheric entry. We examine the possibilities for terrestrial Bi contamination of CP aggregate W7029*A but judge them unlikely. Enrichment of elemental Bi within components of extraterrestrial materials is consistent with a nebula condensation model⁶ and implies that Bi within CP aggregate W7029*A may have formed at a late stage of the condensation process.

W7029*A was collected from the stratosphere as part of the Cosmic Dust Program at Johnson Space Center⁷. Before allocation, a small ($< 10 \mu\text{m}$) fragment of W7029*A was removed from the collection surface for preliminary examination. Scanning electron microscopy (SEM) and bulk chemical analysis by the Cosmic Dust Preliminary Examination Team⁷ indicate that aggregate properties follow the criteria for a typical CP aggregate⁴. Portions of the aggregate were transferred from the collection surface to holey carbon films mounted on Be grids and gently washed with hexane in order to minimize contamination by the silicone oil used in the collection process. CP aggregate W7029*A is fragile and readily disaggregated on removal from the collection surface. Thus, individual fragments of the aggregate were suitably dispersed on carbon films for detailed AEM investigations. Electron microscopy on the allocated sample was performed with a modified JEOL 100CX AEM and with a Phillips EM420 AEM. Accelerating voltages of 100 and 120 kV, respectively, were used for all samples analysed. Each individual fragment or grain from this portion of the aggregate was then identified by a combination of high-resolution imaging, electron

diffraction, electron energy-loss spectroscopy or energy dispersive spectroscopy (EDS). Modal analysis of CP aggregate W7029*A indicates that the following phases are predominant: carbon-rich phases (~47%), layer silicates (~11%), other silicates (~12%) and metals and/or oxides (~8%). Nine Bi grains (comprising ~50% of the metals or oxides) have been identified.

A typical example of a Bi grain from CP aggregate W7029*A is shown in Fig. 1. This conventional transmission electron microscopy (TEM) image shows an angular grain, ~350 × 400 nm in size, with a small (<2 nm) rim of less electron-dense material. The nature of the rim is unknown, but thin rims of a similar nature have been observed on individual crystals of cohenite⁵ and (Fe, Ni) carbides⁸ in other CP aggregates collected from the stratosphere. All Bi grains show a narrow size range between 0.5 and 0.25 μm. EDS analyses of all grains show the characteristic *M* and *L* lines for secondary X-ray fluorescence of Bi (Fig. 2). Determination of Bi from EDS spectra within the 0–10 kV range is difficult due to overlap of the Bi *M* line with the *K* line for sulphur (ref. 9). However, the higher energy-line positions (between 10 and 20 kV) and intensities ($L\alpha > L\beta > L\gamma$) provide an unambiguous identification of Bi. The inset in Fig. 2 shows the lower energy end of an EDS spectrum taken with a windowless energy dispersive spectrometer. A small peak for the oxygen *K* line is evident. The intensity of the O peak is significantly higher than that observed in spectra from carbon substrates containing residual silicone oil. The lower intensity of the O peak relative to the Bi *M* line is probably due to self absorption of the soft oxygen X rays.

Electron diffraction (inset, Fig. 1) from all Bi grains indicates that they are single crystals and confirm the oxide composition from EDS data¹⁰. Bi metal forms in space group $R\bar{3}m$ with cell dimensions $a = 4.537$, $c = 11.838$ Å. Bi forms two oxides, Bi_2O_3 and Bi_2O_5 , the former occurring as three well defined polymorphs¹¹: monoclinic, tetragonal and body centred cubic. Two other polymorphic forms of Bi_2O_3 have also been described: oxide (I) and oxide (II)¹². The structures of these latter forms are not well determined, but oxide (I) has a simple cubic structure with $a = 5.65$ Å (refs 12, 13). All grains observed in CP aggregate W7029*A index according to this simple cubic cell for Bi_2O_3 (ref. 12).

The geochemical properties of elemental Bi are unique and provide a convenient set of independent criteria to argue against terrestrial contamination of CP aggregate W7029*A during its entry into the stratosphere or after collection. Terrestrial Bi may enter the stratosphere by natural or anthropogenic processes. However, we shall present arguments which show that, at the time, neither of these processes was significant for the collection of CP aggregate W7029*A.

Bi is not abundant in the terrestrial crust (ranges between 0.1 and 0.2 p.p.m.)¹⁴ and is commonly found in the native state with traces of As, S and Te (ref. 15). Bi can also be found in sulphide minerals in veins associated with Ag–Pb ore deposits or in replacement deposits formed by hydrothermal solutions¹⁵. Although Bi minerals occur in pneumatolytic deposits associated with volcanic action, the Bi content of volcanic plumes is not known with any degree of certainty. Nevertheless, the collection period for flights which sampled W7029*A does not correlate with any reported volcanic eruption, still less a volcanic plume which penetrated the Northern Hemisphere tropospheric–stratospheric boundary. Thus, the lack of sulphur or other commonly associated elements within these Bi grains and the low concentrations of Bi minerals in the Earth's crust argue against a natural terrestrial origin for Bi in W7029*A.

Anthropogenic Bi contamination may occur through infall of debris from higher altitudes¹⁶, atmospheric nuclear explosion plumes, or at a later stage of the collection and curation process. Alloys and oxides of Bi are used in the space and materials science industries¹⁷. However, in the most common applications (cosmetic and medicinal preparations) Bi is not used in great volumes, and even in low melting temperature alloys the metal is consumed in small quantities¹⁵. Bi within W7029*A derived from either of these sources should show trace or remnant

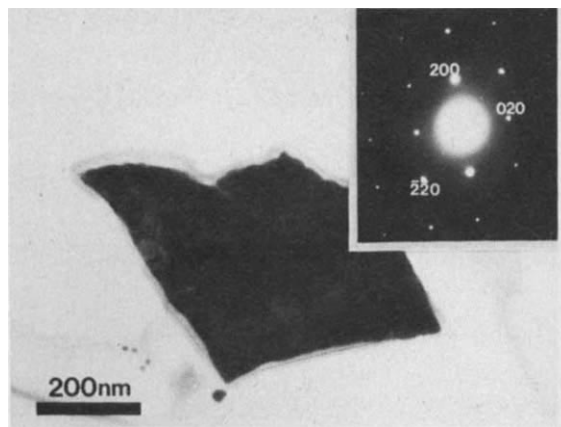


Fig. 1 TEM image of a typical Bi grain (mounted on holey carbon film) from CP aggregate W7029*A. The electron diffraction pattern (inset) is indexed according to a simple cubic form of Bi_2O_3 .

compositions characteristic of the relevant alloy or oxide used in space or on Earth. Such compositional variety is not observed for any Bi particle in CP aggregate W7029*A. Furthermore, a survey of the collection and curation procedures at Johnson Space Center indicates that Bi metals, oxides or alloys are not contaminants at any stage of the process. An extensive survey of all possible forms of contamination within our sample preparation and analysis procedures has been undertaken during the past 2 yr. This survey has included detailed mapping of TEM grids before and after supply to the Curatorial Facility, SEM and AEM analysis of all forms of plastic, epoxy and mounting materials. Grains containing Bi have not been observed within these potential laboratory contaminants or unusual non-chondritic stratospheric particles.

Bi has one naturally occurring isotope (^{209}Bi) with a half life of 2×10^{18} yr (ref. 15). Five other isotopes of Bi occur as the daughter products of uranium or thorium decay. However, the longest half-life for any daughter product from these decay chains is ~5 days (ref. 15). The most recent recorded atmospheric nuclear test in the Northern Hemisphere occurred in late 1974^{18,19}. Artificial tritium fallout studies at the South Pole indicate that stratospheric half residence times for gaseous species (for transport from the Northern Hemisphere to the South Pole) is ~20 months. Therefore, we infer that particulates will remain in the Northern Hemisphere stratosphere in normal conditions for less than 20 months. Any daughter Bi from a nuclear explosion plume in the Northern Hemisphere would have decayed and been transported from the stratosphere by the time CP aggregate W7029*A entered the stratosphere. If the aggregate itself was a product of an atmospheric nuclear explosion, this set of observations would be inconsistent with the present consensus of opinion in the field of interplanetary dust^{1–5}. In addition, transport times¹⁹ and settling rates for stratospheric particles of irregular shape²⁰ do not support a nuclear blast origin for CP aggregate W7029*A.

Terrestrial Bi from natural or anthropogenic sources may contaminate CP aggregates within the stratosphere during the limited time they settle from rest velocity height to the collecting altitude. (This time period is calculated²⁰ to be <150 days.) In addition, for a terrestrial origin of Bi, particle clustering must be shown to be significant during the period of collection of CP aggregate W7029*A. Clustering of particles is primarily dependent on the relative concentration of particles within the stratosphere and is significant for anomalous conditions such as the El Chichón stratospheric cloud²⁰. However, during normal collection periods, particle concentrations within the stratosphere are significantly lower than during the collection period through the El Chichón cloud^{20,21}. Evidence for particle clustering during

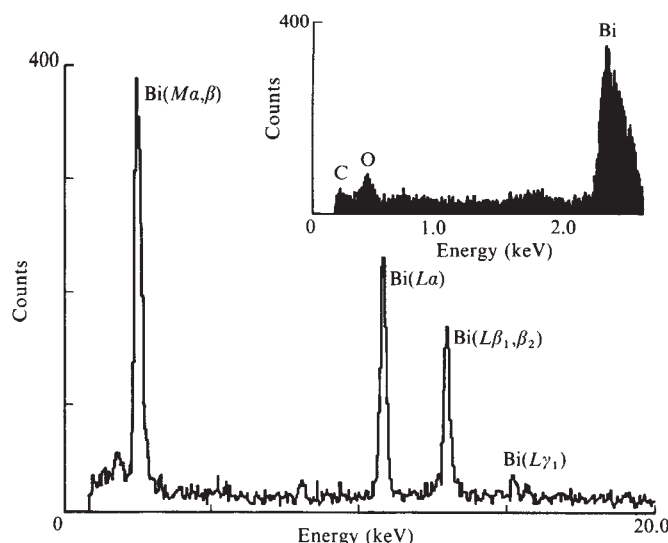


Fig. 2 Energy dispersive spectra from the grain shown in Fig. 1. Only Bi *M* and *L* lines are present with a conventional Be window detector. The windowless energy dispersive spectrum (inset) reveals a significant oxygen peak above background from the substrate. See text for details.

ambient stratospheric conditions has never been reported since the inception of the flat-plate collection process. We believe that particle clustering can occur in these conditions, but in very limited circumstances. These circumstances would primarily involve the most abundant stratospheric particles (such as Al spheres and fragments from solid rocket fuels^{22,23} and would tend to include the abundant smaller size fractions^{24,25}. Al-rich spheres or fragments from rocket debris have not been observed in the complete W7029*A aggregate, nor in any portion allocated for this study. Therefore, we suggest that particle clustering (and contamination by terrestrial Bi grains) is not significant for CP aggregate W7029*A.

Bismuth oxide may form from elemental Bi in a variety of conditions involving heating and oxidation. Throughout the lifetime of CP aggregate W7029*A, major periods of heating may have occurred during interplanetary passage or on entry into the Earth's atmosphere. During the latter process, oxidation may also occur. An understanding of the formation conditions for Bi₂O₃ and probable temperature profiles for micrometeoroids in Earth-crossing orbits may place constraints on the transformation of elemental Bi to Bi₂O₃ within CP aggregate W7029*A.

Fraundorf²⁶ has used typical entry parameters for micrometeoroids in Earth-crossing orbit to calculate temperature profiles for different size particles at different densities as they enter the stratosphere. Although the model does not strictly apply to CP aggregate particles, we use this formulation to estimate that a 60-μm size CP aggregate may have experienced maximum temperatures between 800 and 950 °C for a few seconds during atmospheric passage to 20 km altitude. Fraundorf's model²⁶ does not account for the complex shapes and heat capacities inherent in a typical CP aggregate. Calculations are based on an aggregate size ~60 μm and bulk densities ranging between 0.5 and 1.0 g cm⁻³. Aggarwal and Goswami¹² have produced the simple cubic form of Bi₂O₃ by rapidly heating Bi film in air or in partial vacuum (~10⁻² atm) between 250 and 300 °C. Oxides formed at higher temperatures (using a Bunsen burner flame) melted during oxidation¹². This method for simple cubic Bi₂O₃ formation is kinetically favourable and suggests that the Bi grains in CP W7029*A were heated to ~300 °C during atmospheric entry. The apparent absence of tetragonal Bi₂O₃, which forms at higher temperatures (~900 °C; ref. 11), suggests that the range of temperatures experienced by Bi grains on entry is between 300 and 900 °C. Interpretation of data from associated minerals within this aggregate²⁷ indicates that many particles did not

experience temperatures as high as 800 °C. We have shown that on average, temperatures on entry for CP aggregate W7029*A did not exceed 400 °C (ref. 27). These results are consistent with a known mechanism and our temperature estimate for heating Bi grains on atmospheric entry. Limited periods of pulse heating may also occur during interplanetary orbit, but with less certainty and a lower probability of oxidation. Therefore, we believe that CP aggregate W7029*A contained elemental Bi which transformed to Bi₂O₃ by pulse heating to 300–400 °C on entering the Earth's atmosphere.

In cosmochemistry, Bi is important because it is the heaviest stable element and it is highly volatile. Bi is present in variable, trace concentrations in meteorites and is most abundant in carbonaceous chondrites²⁸. Individual meteorites from other groups also show high abundances of Bi (for example, the unequilibrated ordinary chondrite (UOC) Meso-Madaras²⁸) though, in general, the average bulk analysis for each group is less than carbonaceous chondrites. Individual grains of Bi or Bi₂O₃ have not yet been observed in any meteorite. All previous attempts to determine the presence of Bi within meteorites have used optical microscopy, electron microprobe or bulk chemical techniques. These techniques cannot provide the same spatial resolution as AEM and therefore do not allow direct mineralogical comparisons between CP aggregate W7029*A and Bi-rich meteorites. With further AEM studies, it may be possible to identify the form and nature of Bi minerals in meteorites. Nevertheless, we use bulk meteorite data and current theories of Solar System evolution to provide some insight on the possible cosmochemical significance of Bi within CP aggregate W7029*A.

The unique properties of Bi have been used to predict its relative abundance within meteorites using a two-component model for condensation from a nebula gas⁶. The two-component model correctly predicts the mean abundances of Bi in carbonaceous chondrites (relative to Cl chondrites)^{6,29,30}. This fractionation model has also been used to calculate accretion temperatures for ordinary chondrite meteorites^{29–31}, based on the abundances of volatile elements including Bi. In addition, the predicted siderophile nature of Bi in nebula conditions³¹ has been confirmed by a study of Bi distribution in kamacites in the UOC Kohar³². (Note that both Meso-Madaras and Kohar are mildly metamorphosed Class II, L3 ordinary chondrites³³.) A material termed 'mysterite' has been proposed for ordinary chondrites with unusually high Bi abundances (such as Suphee) and low petrological type³⁴. Mysterite is associated with dark clasts within these chondrites and occurs as a Ti-poor or a Ti-rich variety³⁴. Higuchi *et al.*³⁴ suggest that these two components formed as late condensates from a solar nebula that collected volatiles which were left behind by earlier generations of chondrites. Independent evidence that these processes may occur in the region of the solar nebula sampled by these clasts has been provided by Davis *et al.*³⁵.

The agreement between theory and observations for the nebula condensation of Bi within the frame of reference provided by chondritic meteorites suggests that the implicit assumptions made by Larimer³¹ for two-component model calculations may also apply to other primitive materials within our Solar System. The absolute age of CP aggregates has proved difficult to determine experimentally. CP aggregates may prove to have a cometary origin⁴, and thus, some individual aggregate grains may have formed during our early Solar System evolution³⁶. For the purposes of this study, we assume that CP aggregates are potentially primitive extraterrestrial materials, that Bi will occur in primitive materials as a metal and that the condensation temperature for Bi at nebula pressures between 10⁻³ and 10⁻⁶ atm is between 180 and 225 °C (ref. 31). To estimate the minimum abundance value for Bi within CP aggregate W7029*A, we assume the bulk density of the aggregate is 0.5 g cm⁻³ and that its mass is 10⁻⁸ g. Using these values, we estimate the abundance of Bi within CP aggregate W7029*A to be at least in the p.p.m. range. This value for Bi suggests a considerable localized enrichment relative to cosmic abundance values³¹. Enrichment of Bi within components of extraterrestrial

materials is consistent with a nebula condensation model⁶ and implies that Bi within CP aggregate W7029*A formed at a late stage of the condensation process.

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Occurrence of oxidized components in Qingzhen enstatite chondrite

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Clues to the early history of the Solar System can be acquired by studying the properties of highly unequilibrated chondrites. These chondrites are thought to preserve an almost unaltered record of nebular processes^{1–3}. Here we show that the most unequilibrated enstatite chondrite⁴, Qingzhen, contains a population of enstatite grains which appear to have formed under more oxidizing nebular conditions than the bulk of the meteorite, which is highly reduced. These grains are black in transmitted light because of the presence of micrometre-sized inclusions of Ni-poor, Cr-rich metal and occur either isolated within the matrix or in chondrule interiors. Even though subsequent *in situ* reduction has led to the nucleation of metal, in most cases the FeO content of the enstatite grains still falls above the range found in the reduced portion of the meteorite. The textural occurrence of these grains argues against their having been introduced during collision of Qingzhen with an oxidized meteorite. Most likely, they originated in the same general nebular neighbourhood as the reduced bulk Qingzhen material and were subsequently transported into the reducing environment either before or during the process of chondrule formation. The discovery of this once-oxidized material in Qingzhen poses significant constraints on the existing models of formation of reduced matter in the Solar System.

The enstatite chondrites are characterized by an assemblage of highly reduced mineral phases^{5–7}. Elements which are predominantly lithophilic in other chondrite groups, display in enstatite chondrites, chalcophilic or siderophilic properties. Part of the Si is present in solid solution in the metal or as the Ni-Fe silicide perryite, and the bulk of the Fe occurs as the metal kamacite and the sulphide troilite. The ferromagnesian silicates (low-Ca clino- and orthopyroxene) have very low FeO contents. The FeO/(FeO + MgO) ratio in these minerals ranges from 0.0004 to 0.1, in contrast to values of 0.15 to 0.30 found in the olivine and pyroxene of ordinary chondrites^{7,8}.

The Qingzhen meteorite contains well defined chondrules and chondrule fragments surrounded by a population of metal,

sulphide and enstatite grains. Some of the latter are black in transmitted light and have an average FeO content three to five times higher than enstatite in the chondrule interior⁹, indicating formation under more oxidizing conditions than the bulk of the Qingzhen meteorite.

Sample A is an 80 × 120 μm black enstatite grain which contains fine-grained metallic inclusions (Fig. 1a) oriented sub-parallel to the crystallographic planes of the mineral. An enlarged portion of sample A (Fig. 1b) shows that the metallic inclusions tend to form small clusters or coalesce into larger grains surrounded by optically metal-free enstatite. Glass inclusions are also present. The composition of the enstatite in the metal-free areas is given in Table 1 (A1). The rather high total of this analysis (101.87) indicates an excess FeO (~2%) due to the presence of ultrafine metallic inclusions. The metal particles consist of almost pure Fe, and are Cr-rich and Ni-poor (Table 2, A4) relative to the average metal composition in the Qingzhen matrix. Nickel-poor metal can be produced during reduction of ferrosilite (FeSiO₃) originally present in solid solution within the pyroxene. The reduction reaction: FeSiO₃ → Fe⁰ + SiO₂ + 1/2 O₂ produces free SiO₂. Inclusions of SiO₂-rich glass are found within the enstatite (Table 1, A3).

An approximate estimate of the FeO content of sample A before reduction was obtained by means of broad beam analyses of large areas of the grain (Table 1, A2), assuming absence of pre-existing metal and no metal loss after reduction. The analytical techniques used in this study do not allow for resolving the amount of Fe present as metal from that present as FeO within the enstatite. The bulk Fe present in the area analysed, given as FeO, is 8.6%.

In sample B a set of coarse metal grains is oriented along one of the crystallographic planes of the enstatite and the areas immediately adjacent to the coarsest grains are relatively free of metal (B1, Table 1). The original FeO content of the grain, obtained by broad beam analysis, is 9.3% (B2 in Table 1). Glass inclusions are present, but are too small to be analysed. The metal (B3, Table 2) has higher Cr and lower Ni contents than the metal within Sample A.

Sample C (60 × 80 μm) consists of a fine-grained intergrowth of metal, enstatite, glass and sulphide, surrounded by clear enstatite (Fig. 1c). The reduction process was accompanied by a significant degree of melting followed by crystallization of clear enstatite (Fig. 1d). Broad beam analyses of optically metal-poor areas within the intergrowth (C1, Table 1) reveal a high FeO content (17.3%) in spite of only a slightly high total

Table 1 Chemical data on oxidized components in Qingzhen (in weight %)

	Sample A			Sample B		Sample C			Sample D		
	A1	A2	A3	B1	B2	C1*	C2	C3	D1†	D2	D3
SiO ₂	56.6	58.1	79.3	57.9	56.2	51.1	59.7	63.8	55.1	59.0	98.9
Al ₂ O ₃	—	—	5.1	—	—	—	—	14.7	2.0	—	—
Cr ₂ O ₃	0.73	0.90	0.38	0.71	0.78	1.32	—	—	0.91	—	—
CaO	0.27	0.13	1.22	—	—	2.3	—	—	0.86	0.23	—
MgO	39.2	36.8	10.9	38.9	38.0	29.9	40.5	7.7	33.8	39.0	—
FeO	4.9	8.6	2.9	2.4	9.3	17.3	1.07	1.4	7.6	1.76	1.10
Na ₂ O	—	—	1.92	—	—	—	—	9.9	—	—	—
Total	101.70	104.53	101.72	99.91	104.28	101.92	101.27	97.50	100.27	99.99	100.00

* Also MnO (0.41%) and S (0.8%).

† Also S (0.35%).

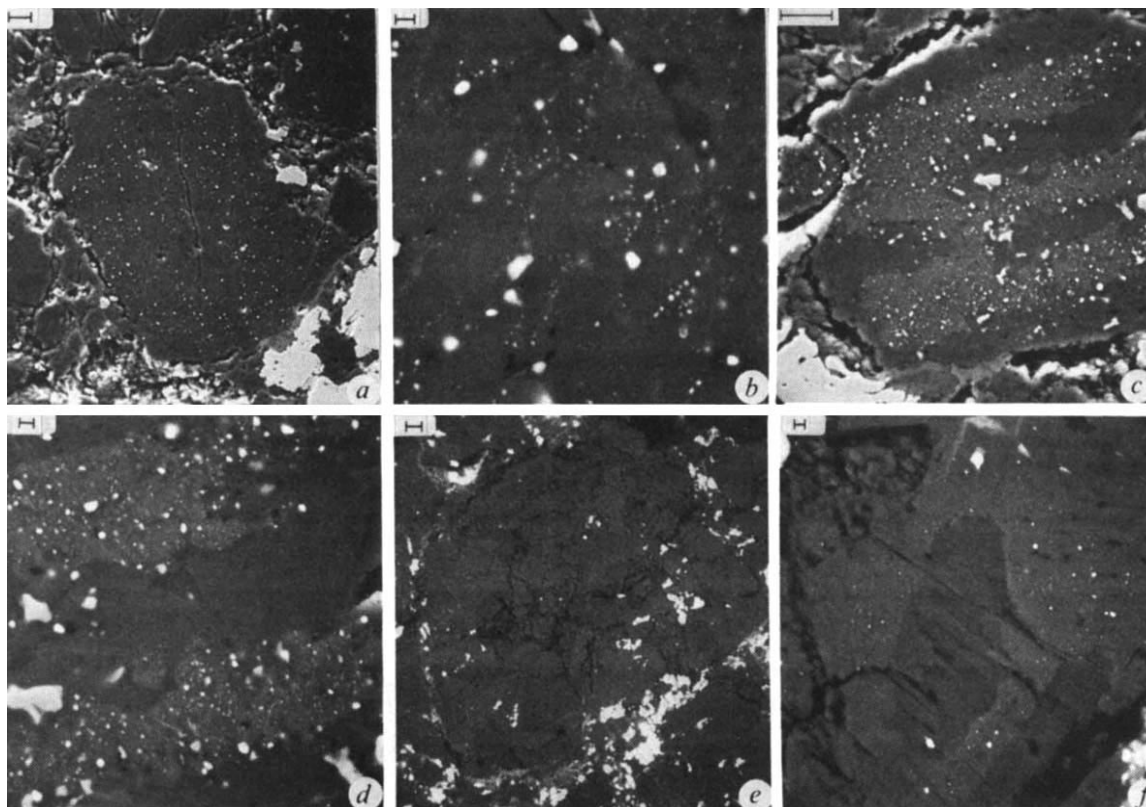


Fig. 1 *a*, A 80×120 μm grain of black enstatite (sample A) in Qingzhen, containing inclusions of Ni-poor metal, oriented subparallel to the crystallographic planes of the mineral. *b*, Enlargement of a portion of sample A, showing clustering and coalescing of the metallic particles. SiO₂-rich glass inclusions (dark gray) are also visible. *c*, Sample C (60×80 μm) consists of fine-grained intergrowth of metal, enstatite, glass and sulphide, surrounded by inclusion-free enstatite. *d*, Enlargement of sample C, showing textural evidence that melting has occurred. *e*, A 150×200 μm chondrule fragment (sample D) consisting of enstatite (light grey) and tridymite (dark grey). *f*, An enlargement of a portion of sample D showing that the enstatite contains sub-micrometre inclusions of metal and vugs. Metal and vug-free enstatite (medium grey) has crystallized in the interior of SiO₂-rich glass areas. Scale bars: *a*, *c*, *e*, 10 μm; *b*, *d*, *f*, 1 μm.

(101.92%). The clear enstatite (C2) has a low FeO content and is Cr₂O₃- and CaO-free. Droplets of glass of prevalently albitic composition (C3, Fig. 1*d*) have segregated into the clear enstatite, suggesting the presence of a feldspathic component within the metal-rich intergrowth. Most metal is Ni-poor (C4, Table 2), but a few grains of Ni-rich metal have been found (C5) indicating that some metal was originally present within the precursor assemblage.

Sample E is a 150×200 μm chondrule fragment consisting of enstatite and tridymite in an approximately 1:1 ratio (Fig. 1*e*). The enstatite crystals contain sparse sub-micrometre metal inclusions and abundant vugs (Fig. 1*f*) suggesting loss of a volatile component during chondrule formation. SiO₂-rich glass included within the pyroxene is coated with clear enstatite which is vug-free (Fig. 1*f*). The enstatite has a high FeO content (7.6%) and contains significant amounts of Al₂O₃, Cr₂O₃ and CaO (D1, Table 1). This is the highest FeO content ever reported in

pyroxenes from enstatite chondrites. It is about 10 times higher than the FeO content of enstatite within 'reduced' chondrules of Qingzhen. The clear enstatite is FeO-poor and Al₂O₃- and Cr₂O₃-free (D2) and the tridymite contains only minor amounts of FeO (D3). The metallic inclusions within the enstatite are too small to be analyzed, but metal grains, interstitial among enstatite and tridymite, are Ni-rich (D4, Table 2). The high FeO content of the enstatite, and the small number of metal inclusions strongly suggest that only minor reduction has occurred within this chondrule.

Qingzhen also contains a population of porphyritic chondrules containing grains of olivine with micron-size inclusions of metal^{10,11}. The metal is Ni-poor and was probably produced by *in situ* reduction of the fayalitic (Fe₂SiO₄) component of the olivine¹². The original fayalitic content of the grains (15–25 mol %) is inconsistent with the composition of materials formed under strongly reducing conditions.

Table 2 Chromium and Ni content of metal within matrix and oxidized components in Qingzhen (in weight %)

	Matrix	A4	B3	C4	C5	D4
Cr	<0.05	1.94	4.4	0.96	0.50	0.50
Ni	3.4	0.95	0.60	1.02	4.1	5.4

Ni-poor metal might represent the condensation product of Fe vapour produced during shock reheating and introduced within the enstatite grains along cracks and crystallographic planes (D. W. Sears, personal communication). As discussed by Sears^{13,14}, the vapour to form first when Fe, Ni alloy is evaporated is Ni-free because of the lesser volatility of Ni. As shown in Table 2, the Ni-poor metal contains substantial amounts of Cr, while the matrix metal is Cr-free. Therefore, evaporation of the latter could not produce a Cr-rich vapour. Furthermore, the metallic inclusions occur within enstatite grains which in most cases still retain FeO contents significantly higher than the 'reduced' enstatite in the surrounding matrix and chondrules. Incomplete reduction of precursor oxidized silicates appears to be the only possible mode of formation of the Ni-poor metal.

The observed range of FeO contents in the ferromagnesian silicates of Qingzhen (0.15–7.6%) represents a lower limit, because reduction has occurred. The highest calculated FeO content is 18% for relict olivines inside incompletely melted chondrules. Even though the bulk of the Qingzhen meteorite is highly reduced, such high ranges of FeO contents are comparable to those observed in highly unequilibrated ordinary chondrites.

We exclude the possibility that the oxidized components in Qingzhen were introduced during collision with a more oxidized body and subsequently reduced. These components occur as single grains or chondrules surrounded by highly reduced material, not as xenolithic clasts. Furthermore, the presence of FeO-rich enstatite in chondrule interiors indicates that these components were already present prior to or during the process of chondrule formation, and therefore long before the accretional event that culminated with the formation of the parent body of Qingzhen.

Various reheating mechanisms could have triggered the reduction process and the melting (often incomplete) of these oxidized components. Reheating during shock is a possibility. Wang and Xie¹⁵ describe some features in Qingzhen which are suggestive of shock. But shock features are absent in the coarse grained sulphide minerals in the matrix, which are always monocrystalline, and not polycrystalline as one would expect had they been shocked (A. El Goresy, personal communication). Thermal metamorphism in the parent body interior is another possibility. This process, which is recorded in the texture and chemistry of ordinary chondrites of increasing petrologic type, brings about mineral recrystallization and equilibration leading ultimately to mineral phases with uniform FeO/(FeO/MgO) ratios. But thermal metamorphism cannot be responsible for the reduction of the oxidized components in Qingzhen for the following reasons: (1) Qingzhen is highly unequilibrated and contains neither the textural nor the chemical evidence of metamorphic processes; (2) in many cases the grains were accompanied by melting, while the bulk of Qingzhen was never melted.

Thus, a reheating event outside the parent body is required, leaving the chondrule formation process as the only plausible alternative. It has been recently shown that this process may lead to incomplete melting of chondrule precursor assemblages and reduction during chondrule formation has been well documented in highly unequilibrated ordinary chondrites^{2,12,16}. We suggest that oxidized ferromagnesian silicates occurring either isolated or as clusters of grains were reheated and melted to various degrees during the chondrule formation event that produced the main population of highly reduced chondrules in Qingzhen.

The less reducing nebular conditions required for the stability of the oxidized components found in Qingzhen could be obtained by assuming local fluctuations in the degree of reduction of the nebular gas possibly related to inhomogeneous admixture of a reducing component.

It has been recently suggested that the onset of the reducing conditions required for the formation of enstatite chondrites may be produced by loss of O-bearing compounds, in the form of high temperature condensates (refractory compounds of Ca, Al and Ti)¹⁴, or by a process of dust-gas fractionation followed by evaporation and condensation in the dust-(and O) depleted nebular region¹⁷. According to these models, failure of the residual solids to equilibrate with the nebular gas or incomplete evaporation of solids in the dust-depleted region, could allow the preservation of oxidized material. Alternatively, the oxidized components found in Qingzhen might represent materials originated in dust-enriched nebular regions¹⁷, which were subsequently transported into an adjacent more reducing environment.

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Presence of a superparamagnetic component in the Orgueil meteorite

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The Orgueil CI carbonaceous chondrite meteorite fall of 14 May 1864 is of particular interest because of the presence of an organic component which included amino acids. Using Mössbauer spectroscopy, we have determined that the Orgueil meteorite contains an appreciable fraction of its iron in the superparamagnetic state. This result implies that the superparamagnetic iron component was formed during an aqueous phase in the history of the parent body, and that the superparamagnetic component may have served as a catalyst for formation of the organic component.

A 52.4-mg sample from the Smithsonian National collection (Orgueil USNM-234) was ground into a powder and dispersed into 78.4 mg of molten paraffin. On cooling, the paraffin was moulded, while plastic, into a 6-mm diameter pellet that was then supported by a 25-mm lead annulus. The sample was cooled to temperatures between 21 and 295 K by transferring heat by gaseous helium from the sample to an Air Products closed-cycle refrigerator. This method of heat transfer was used to prevent mechanical vibration of the sample by the closed-cycle refrigerator. The Mössbauer spectrometer employs a liquid-nitrogen cooled Si(Li) detector for the 14.4-keV ⁵⁷Fe γ rays.

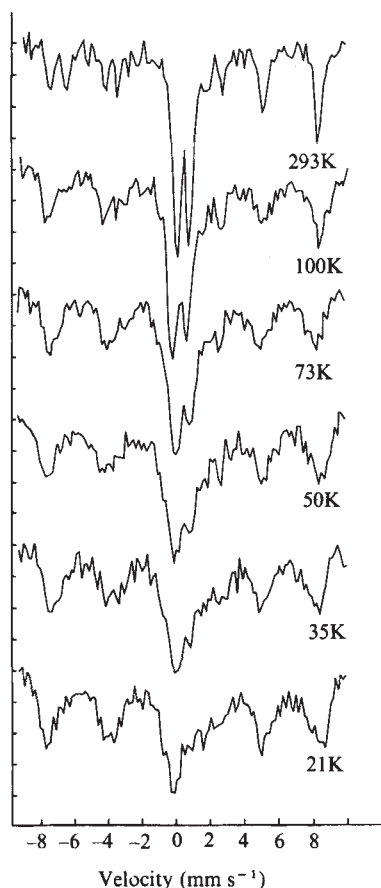


Fig. 1 Mössbauer spectra of the Orgueil CI carbonaceous chondrite. The 293 K spectrum shows the presence of ferromagnetic magnetite and two paramagnetic absorptions near 0 mm s⁻¹. Spectra obtained below the Verwey transition at 119–116 K, at 100 K, 73 K, 50 K, 35 K and 21 K show a gradual diminishing of the paramagnetic absorption and enhancement of the ferromagnetic absorptions indicating the presence of a superparamagnetic magnetite component.

A spectrum was obtained at 295 K in order to characterize the sample. This spectrum, similar to that obtained by Gerard and Delmelle¹, shows a blending of paramagnetic features with the ferromagnetic spectrum of magnetite². Subsequent measurements were made at temperatures of 100, 73, 50, 35, and 21 K. This range is below the Verwey transition that occurs in Fe₃O₄ between 119 and 116 K, and was chosen because previous studies³ have shown that the Fe₃O₄ ferromagnetic spectrum does not alter between 100 and 7 K. Each spectral measurement required ~5 days of counting because of sample size and source strength. The spectra obtained between 100 and 21 K show the same gradual diminishing of the paramagnetic absorptions and enhancement of the ferromagnetic features as observed for superparamagnetic materials where the particles are of sub-domain size⁴⁻⁶.

Such particles are in a state of uniform magnetization with a net magnetic moment possibly 10⁵ times that of a single atom (hence 'superparamagnetic'). For sufficiently small particles at higher temperatures, the relaxation time of the magnetic moments of these particles is smaller than the nuclear Larmor precession time, and the Zeeman splitting collapses to the two-peak spectrum characteristic of the paramagnetic state⁵. As temperature decreases for a given particle size, the relaxation time increases to the point where the Zeeman splitting can be observed (ferromagnetic state). When a distribution of sub-domain particles exists, as is usual, that distribution can be determined from the Mössbauer spectrum^{4,5}. We plan to carry out such determinations in the future. By taking the ratio of the integrated paramagnetic iron absorption between 100 and 21 K

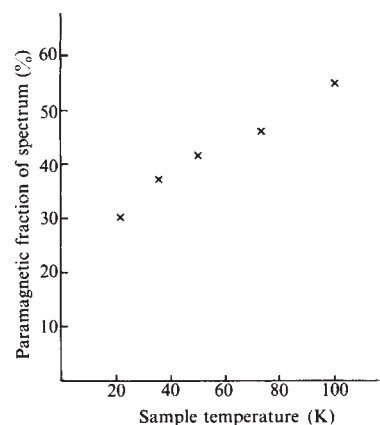


Fig. 2 Paramagnetic fraction of spectrum versus sample temperature. The decrease between 100 and 21 K indicates the superparamagnetic magnetite component held by ~30% of all iron.

and the total integrated absorption of all forms of iron, we estimate that ~30% of the iron of the meteorite is in the superparamagnetic subdomain form. The remaining iron is ~45% ferromagnetic and 25% paramagnetic. The magnetite is thus ~40% superparamagnetic and 60% ferromagnetic. Characteristically, superparamagnetic particles are <300 Å in size, which indicates that the Orgueil contains an appreciable portion of iron as Fe₃O₄ in this size range. The magnetite content of the Orgueil meteorite has been determined using the Faraday balance and the vibrating sample magnetometer to 11.0 ± 0.4 wt% (ref. 7). Combined with the present results, this suggests the superparamagnetic magnetite component is ~4.5 wt%.

The presence of a superparamagnetic component in the Orgueil meteorite requires a discussion of its origin and its impact on the origin of the organic component. Particles having sizes in the <300 Å range are almost certainly not the result of size reduction processes. Superparamagnetic particles can be produced by precipitation from aqueous solutions⁵, vapour condensation⁸, and crystallization from a melt⁶. The last mechanism is unlikely for a CI carbonaceous chondrite such as the Orgueil. There is evidence for aqueous activity in the parent body of the Orgueil meteorite⁹⁻¹¹, as well as those of other carbonaceous chondrites. The magnetite superparamagnetic particles could be the result of precipitation from an aqueous solution or they may be a secondary product arising from the oxidation of iron sulphides in aqueous conditions, although oxidation in the solar nebula is also a possibility⁷.

While aqueous processes are those most likely to be responsible for the superparamagnetic magnetite component of the Orgueil meteorite, either directly through precipitation or indirectly through oxidation of iron sulphide, an alternate hypothesis for superparamagnetic particles has been put forward by Cameron¹². He argued that superparamagnetic magnetite relict cores of interstellar grains, such as proposed by Jones and Spitzer¹³, might be found in carbonaceous chondrites of type I. This hypothesis needs to be re-examined in the light of the result presented here. It may be that the Orgueil superparamagnetic component is a solar nebula analogue to interstellar grain cores.

The possibility that catalytic activity of magnetite in meteorites and in the solar nebula is responsible for meteorite organics has been reviewed by Anders *et al.*¹⁴. The organic component of the Orgueil includes amino acids¹⁵, fatty acids¹⁶, alkanes¹⁴, heterocyclics¹⁷, and polymeric material¹⁸. Nickel-silica catalysts prepared by coprecipitation and exhibiting superparamagnetic properties^{19,20} are utilized in laboratory and industrial organic synthesis. A superparamagnetic magnetite component would thus be an attractive candidate as a catalyst for the formation of organics in the Orgueil meteorite.

A preliminary examination of bulk samples and separated magnetic fractions of the Allende meteorite has not revealed any superparamagnetism in the iron minerals. The bulk of the iron was paramagnetic and only a small separated fraction

exhibited ferromagnetism. Sears and Marshall²¹ have pointed out that while Mössbauer spectroscopy does not reveal magnetite in the Tieschitz unequilibrated ordinary chondrite²², X-ray diffraction indicates its presence. The difference between those results may be due to the magnetite in the Tieschitz being entirely superparamagnetic, and hence not appearing in the expected ferromagnetic absorption pattern of a 293 K Mössbauer measurement, while X-ray diffraction would show the lattice structure of particles $\ll 300$ Å in size. This question, coupled with the results of our preliminary examination of the Orgueil meteorite at a range of cryogenic temperatures, suggests that the Mössbauer technique has broader applications in meteoritics than previous studies have indicated.

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The ability of small molecules to form clathrate hydrates of structure II

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Since gas hydrates were shown in the mid-1950s¹ to be water clathrates of two distinct crystallographic structures (Table 1), it has been generally accepted that structure I hydrates are formed by molecules with maximum van der Waals diameters of up to about 5.8 Å while structure II hydrates are formed by larger molecules, up to about 7.0 Å in size. No exception to this general rule has been observed among more than 100 individual species of molecules known to form clathrate hydrates^{2,3}. From X-ray and neutron diffraction studies we now find, however, that the two smallest molecules which form clathrate hydrates—argon and krypton—do so in the structure II modification. This finding supports a suggestion of Holder and Manganiello⁴.

One of the reasons for the shortage of crystallographic data for gas hydrates of small guest molecules is because such hydrates are stable near 0 °C only at relatively high pressures.

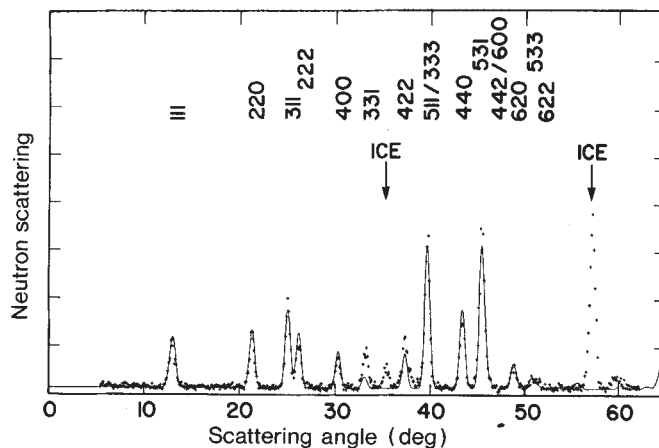


Fig. 1 Comparison of the observed (points) and fitted neutron scattering profile of krypton deuteriohydrate at 5.7 K.

Powder diffraction patterns, however, may be recorded at low temperatures (≤ 100 K) without appreciable hydrate decomposition. For the X-ray and neutron diffraction studies described here, powdered samples were prepared by grinding H₂O or D₂O ice in a rotating rod mill at -30 °C for several days under gas pressures (2 MPa for Kr and CH₄, 10 MPa for Ar) twice as high as required for hydrate stability.

X-ray powder photographs of Ar and Kr hydrate samples were taken with MoK α radiation, as previously described⁵. Lines were readily indexed in the structure II cubic Fd3m space group, with observed intensities in good agreement with structure factor calculations which assumed full occupancy of the 12- and 16-hedral cages.

Elastic neutron scattering measurements on Kr and CH₄ D₂O hydrates were made on the C5 spectrometer operated in a two-axis mode at the NRU reactor, Chalk River. The monochromator was Si(111) and the neutron wavelength 2.22374 Å. A sapphire filter in the incident beam and a Si(111) analyser set for elastic scattering were used to reduce the background. The powder diffraction profile of Kr deuteriohydrate at 5.7 K is shown in Fig. 1. Except for two peaks from hexagonal ice the observed peaks are indexed on the basis of space group Fd3m. No peaks from structure I were observed. Integrated intensities were fitted to a structure II hydrate lattice, with all cages filled and zero thermal parameters for O and D. Because of the limited range of scattering angle, the fit was not very sensitive to these two assumptions.

The results are summarized in Table 2. The lattice parameters a found for Ar and Kr hydrates at 100 K are similar to those found for other structure II hydrates^{5,6}, which average less than 0.1 Å larger at comparable temperatures. Methane hydrate is established as structure I. In the hydrates of N₂ and O₂, which require higher pressures for stability than any other known hydrate⁷, the guest molecules are smaller than methane: the structures of these hydrates must now be considered uncertain.

Except for small compressibility effects, the ideal solid-solution theory of clathrate hydrates⁸ gives for the chemical potentials of water molecules in structures I and II

$$\mu(\text{I}) = \mu^0(\text{I}) - \frac{kT}{23} [\ln\{1 + C_S(\text{I})P\} + 3 \ln\{1 + C_L(\text{I})P\}]$$

$$\mu(\text{II}) = \mu^0(\text{II}) - \frac{kT}{17} [2 \ln\{1 + C_S(\text{II})P\} + \ln\{1 + C_L(\text{II})P\}]$$

where μ^0 refers to water in the hypothetical empty lattice, C_S is the Langmuir constant which relates the extent of occupancy by the gas of the small 12-hedral cages to the pressure P (strictly, the fugacity) of the hydrate-forming gas, and $C_L(\text{I})$, $C_L(\text{II})$ are corresponding Langmuir constants for the 14- and 16-hedra. The 12-hedra being similar in the two structures, $C_S(\text{I}) \sim C_S(\text{II})$.

Table 1 Unit cells of cubic clathrate hydrates

	Structure I	Structure II
Cell size a , symmetry	12.0 Å, Pm3n	17.2 Å, Fd3m
No. of water molecules	46	136
No. of 12-hedra, diameter*	2, 5.0 Å	16, 5.0 Å
No. of 14-hedra, diameter	6, 5.8 Å	—
No. of 16-hedra, diameter	—	8, 6.6 Å
Limiting compositions:		
All cages filled	M·5 $\frac{3}{4}$ H ₂ O	M·5 $\frac{2}{3}$ H ₂ O
Only large cages filled	M·7 $\frac{2}{3}$ H ₂ O	M·17 H ₂ O

* Average free diameter of cage.

Table 2 Clathrate hydrates formed by small molecules

Guest/host	Diameter of guest	Structure	X-ray lattice parameter a
Ar/H ₂ O	3.83 Å	II	17.07 ± 0.04 Å (100 K)
Kr/H ₂ O	4.04	II	17.08 ± 0.08 (100 K)
Kr/D ₂ O		II	17.01 ± 0.05 (100 K)
Kr/D ₂ O		II	17.01 ± 0.01 (5.7 K)*
CH ₄ /D ₂ O	4.58	I	11.77 ± 0.01 (5.2 K)*
H ₂ S/H ₂ O	4.58	I	11.96 ± 0.05 (125 K)†
Xe/H ₂ O	4.57	I	11.84 ± 0.02 (110 K)‡

* From neutron diffraction.

† S. R. Gough, unpublished data.

‡ Ref. 12.

The lowest gas pressure P_0 at which a hydrate can exist is the pressure which lowers its chemical potential to that of ice or liquid water at the same temperature: whether structure I or structure II is stabler depends on which has the smaller value of P_0 . Although accurate values of μ^0 are not known, $\mu^0(\text{II}) < \mu^0(\text{I})$ because of the greater similarity to ice of the hydrogen bonding between the water molecules in the structure II lattice².

For relatively large guest molecules (M) structure II is more stable because $C_L(\text{II}) \gg C_S$, $C_L(\text{I})$, only the 16-hedra are occupied by M molecules, and the compositions approach M·17H₂O. For very small guest molecules, on the other hand, structure II stability results from the condition $C_S > C_L(\text{I})$, $C_L(\text{II})$, that is, from a preference for the small cages, and the composition is much different, about M·6H₂O. Structure I will generally be preferred when $C_L(\text{I}) > C_S$, $C_L(\text{II})$ or $C_L(\text{I}) \approx C_S > C_L(\text{II})$. The former condition appears to apply to most structure I hydrates since even for a guest molecule as small as Xe (Table 2) NMR studies have shown⁹ that $C_L(\text{I})$ is an order of magnitude larger than C_S near 0 °C.

The limited success of past calculations of hydrate dissociation pressures from assumed intermolecular potentials^{2,8,10} now appears to be due, at least in part, to the erroneous assignment of structure I to the model hydrates of argon and krypton. Accurate experimental determination of the compositions of Ar and Kr hydrates should prove of importance in the evaluation of $\mu^0(\text{II})$. Values of $C_S(\text{II})$ for Ar and Kr in the double hydrates of CHCl₃ have already been measured by Barrer and Edge¹¹.

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Reflected turbidity currents on an Ordovician basin floor, Canadian Appalachians

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Sedimentary structures associated with turbidity current deposits show either partial or complete Bouma T_{ac} sequences¹ that suggest deposition of sediment from a waning (decelerating) unidirectional flow². Bathymetric highs and basin margins can cause turbidity currents to reflect back along their original flow paths, a rare situation in both ancient^{3,4} and modern⁵ marine basins. The internal structures of such contained, or reflected, turbidites have only been described in one case, the Contessa bed (ref. 4 pp. 268, 300). Our data from 1–6-m thick graded, mud-dominated turbidites from the Quebec Appalachians, using mainly sole marks, megaripple, ripple and grain fabric data, suggest that during the deposition of these beds the turbidity currents were reflected several times from either internal basin highs or basin slopes. The recognition of such beds not only provides an insight into the depositional environment (deep-water basin floor), but also allows detailed bed-by-bed correlations over tens of kilometres of coastal sections since the beds are reliable chronostratigraphical datum lines.

The beds form part of the 1.5-km thick β_1 and β_2 members of the Middle Ordovician Cloridorme Formation⁶, a flysch unit deposited in a constricted foreland basin during the Taconic Orogeny⁷. The β_1 and β_2 sequence consists of thinly-bedded classic turbidites and hemipelagic shales, punctuated about every 10 m by a thick bipartite bed consisting of a lower, graded sandstone (1/4) and a texturally homogeneous, structureless, silty mudstone cap (3/4). These beds range in thickness from 1 to 5.5 m, and can easily be correlated with little apparent thickness change between localities 25 km apart.

Most beds have basal flute casts that indicate flow from east to west. This led earlier workers⁸ to interpret the beds as deposits of unusual, unidirectional turbidity currents. Internal sedimentary structures consist of parallel lamination, cross-lamination, small-scale megaripple and ripple forms, climbing ripple lamination, wavy and convolute lamination, and pseudonodules⁸. Grading in the lower sandstone part is not continuous, but is punctuated by mud-draped partings across which grain size may decrease abruptly. Between these grain-size breaks, or muddy

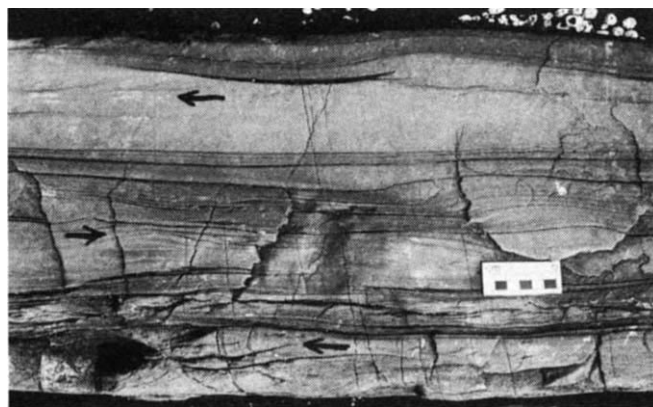


Fig. 1 Sandstone divisions deposited by a reflected turbidity current. The sandstone part of the turbidite is ~30 cm thick. The mudstone cap (not shown) is 105 cm thick (bed PF-2, Fig. 2). Arrows indicate flow directions through the bed.

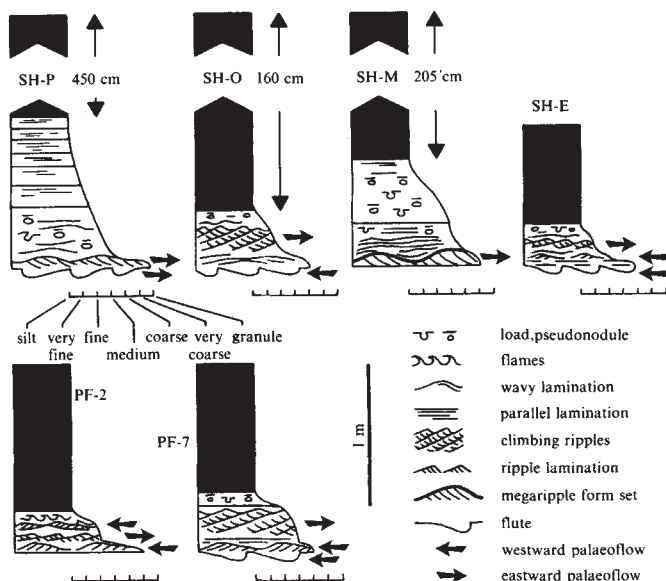


Fig. 2 Sketches of six beds deposited by reflected flows. Structures are accurately drawn to show observed eastward or westward flow.

partings, sedimentary structures or grain imbrication indicate flow from either east to west, or from west to east, with measured palaeoflow azimuths frequently 160° – 180° opposed to one another in the same bed (Fig. 1). At the top of the sandy part of a few beds, alternating divisions of muddy lamination and pseudonodules may repeat several times, each couplet being finer and thinner than the one below. The uppermost sandy division, in all beds, consists of pseudonodules. Sketches of the internal features of six beds appear in Fig. 2. We have observed about 50 such beds in members $\beta 1$ and $\beta 2$.

The form set of cross-stratification at the base of bed SH-M (Fig. 2) has been interpreted as the deposit of antidunes migrating upcurrent^{9,10}, due to the 180° difference between; (1) cross-bed dip direction; and (2) palaeoflow direction indicated by flute casts in surrounding beds. Our discovery of both eastward and westward palaeoflows in single beds removes the original impetus for the antidune hypothesis. In addition, the grain fabric of the cross-strata¹¹ indicates that the depositing current flowed from west to east, opposite to the direction required by the antidune hypothesis. We interpret this form set, therefore, as a mud-draped megaripple train; the megaripples were migrating from west to east.

Each graded bed was deposited by a large volume turbidity current. The thick, poorly-graded mudstone caps suggest flows with enormous amounts of entrained mud. Despite the palaeoflow reversals in the sandy parts of beds, deposition was rapid, as suggested by both abundant climbing ripples and loading (pseudonodules).

We explain the details of sedimentation of these beds as follows (Fig. 3). The large-volume, mud-rich flows were generated along steep, tectonically-formed slopes on the south side of the foreland basin⁶. On reaching the base of the slope, the powerful flows were constrained to flow westward into the area where our sections are now located; most flutes were eroded by this first westward pass of the current. The lowest sand division was either deposited by the initial westward-flowing current, or by the first return flow. There is often a sharp grain-size break above the lower division, followed by a thin mud drape. We believe this sequence indicates: (1) deposition of a coarse basal division; (2) passage of the main flow across the basin floor; (3) suspension fallout of mud from thick, residual, suspended-sediment cloud; and (4) return of the reflected current to deposit the next sandy division. The return flow was always less competent than the first westward flow, due to frictional energy losses and loss of sediment load and, therefore, transported sand significantly finer than that in the lower division (Fig. 2).

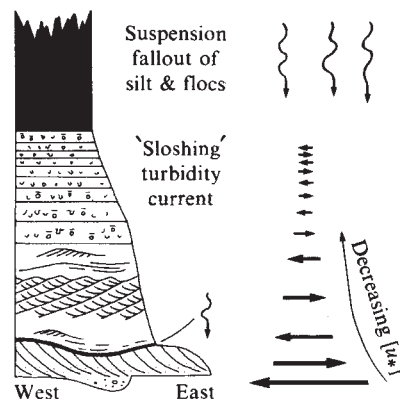


Fig. 3 Depositional model and a representative structure sequence for contained, reflected flows. The length of east-west arrows indicate relative current speeds as the flow decelerates. See Fig. 2 for key.

Several beds have a basal division of coarse to granule grade sand with a fabric or sedimentary structures indicating eastward sediment transport. Included in this category is the bed with basal megaripple cross-stratification (bed SH-M, Fig. 2). These coarse divisions are better sorted than overlying sand, and may not have been deposited entirely by the first reflected current. The sand, due to its coarse grain size, was probably deposited by the initial westward-flowing current, and later reworked as bedload beneath the first reflected flow.

After sand deposition, the ponded, residual, silt-mud suspension was probably still moving slowly back and forth across the basin floor, gradually losing momentum. We believe that it was at this time that the fine-grained pseudonodule divisions were deposited. The silt was deposited quickly, leading to extensive loading and disruption of lamination by liquefaction. In the case of the largest flows (bed SH-P, Fig. 2), the periodic, decaying oscillation of the current led to the deposition of alternating laminated (current deposited) and pseudonoduled (liquefied) layers of decreasing thickness.

The final phase of sedimentation was the fallout of mud from the ponded suspension to form a thick, homogeneous, mudstone cap. The concentrated mud cloud deposited rapidly, perhaps in the same way that shelf muds are rapidly deposited in areas of high concentration, regardless of the competence of local currents¹². Lack of grading in the caps is also consistent with rapid deposition. Clay minerals probably formed particle aggregates (flocs) that settled much more quickly than single particles.

Unlike Bouma-type turbidites, these contained, or reflected, turbidites do not have a predictable sequence of internal sedimentary structures. In particular, ripple lamination, wavy lamination, convolution, pseudonodules and parallel lamination may alternate in an unpredictable way in the upper division of the sandstone part of these beds. Flow direction in these divisions may change one or more times up through a single bed. For each bed, the exact details of deposition depend on: (1) the importance of flow reflection; (2) the distance that the flow travelled before returning to deposit more sediment; and (3) the importance of interference and collisions between separated parts (offspring) of the single primary flow (parent) that could be moving along different paths and, perhaps, overriding one another because of density differences.

Contained, reflected turbidites can, with care, be distinguished from classic Bouma-type turbidites, based primarily on palaeoflow reversals and thick mudstone caps produced by ponding of the residual mud-silt cloud³. These unusual beds are widespread, and provide compelling evidence for deposition in a confined basin. Contained turbidites have only been described from deep-sea sediments, but thinner and perhaps finer-grained examples should also be expected in turbidite sequences of glacial or alpine lakes and arctic fjords with suitable geometry.

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Sampling strategy for stable carbon isotope analysis of tree rings in pine

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Stable carbon isotope measurements in tree rings have been explored as potential indicators of past climate^{1,2}, and as proxy data for reconstructions of the history of $^{13}\text{C}/^{12}\text{C}$ variations of atmospheric carbon dioxide as related to the global carbon cycle^{3–6}. Results of these studies are often conflicting, perhaps partly due to the limited style of sampling natural systems with large inherent spatial variability. We have examined stable carbon isotopic trends in tree rings among several radii of the same tree (*Pinus edulis* Engelm.) and among several trees from the same site, to determine the extent of this variability. The circumferential range in $\delta^{13}\text{C}$ values of cellulose is ~1–1.5%, whereas among individuals it is ~2–3%. Tests to determine how well various combinations of cores fit the trend of an individual tree or of the site as a whole indicate that pooling four cores from four trees accurately represent site $\delta^{13}\text{C}$ trends and absolute values.

Whereas dendroclimatological studies commonly sample several tens of trees at each site with two cores per tree⁷, the

time and expense of carbon isotopic analysis precludes such density of sampling at each site, and often limits analysis to groups of years rather than individual years. The potential weaknesses of single radius (or core) sampling in isotopic studies was revealed by the $\delta^{13}\text{C}$ results of Tans and Mook⁸ for two radii of the same *Quercus rubra* L. cross-section which showed not only different isotopic trends but up to 4% differences between corresponding rings. The factors which influence carbon isotopic fractionation in trees have already been addressed in models such as that of Francey and Farquhar⁹. However, the patterns and magnitude of this fractionation in natural settings have not been discussed in such detail. To test for circumferential variability we obtained two cross-sections from a pinyon pine tree (*Pinus edulis* Engelm.) located at a site 30 km south of Flagstaff, Arizona, cut at the end of the 1981 growing season. To test for intrasite variability we collected cores from eight other *P. edulis* individuals from the same site at the end of the 1982 growing season.

The wood materials were dated at the Laboratory of Tree-Ring Research of the University of Arizona. One cross-section was divided into eight equally spaced radii which were cut out as 'cores' 5 mm wide. The rings were separated into 5-yr groups from 1900–04 to 1975–79, plus 1980–81 for the cross-section samples and 1980–82 for the cores. The ring intervals from the second cross-section were milled from the full circumference. The cellulose (holocellulose) component of the wood was isolated in a method modified after Green¹⁰ (details of preparation and analysis are given in refs 5, 11). The $^{13}\text{C}/^{12}\text{C}$ isotopic ratios are expressed with respect to the international PDB standard¹². Overall reproducibility, including sample preparation, combustion and mass-spectrometric analysis, is estimated to be ~0.1%.

Several studies have reported on circumferential $\delta^{13}\text{C}$ variability in leaves^{11,13,14} and rings^{3,6,8,13,15,16–18}. Despite a variety of species and localities, the range of $\delta^{13}\text{C}$ around the circumference of tree rings is typically 1–2%. Except for the Tans and Mook⁸ results, the isotopic trend from a single core has generally been found to parallel that of others, even though absolute values may differ. From the standpoint of practical sampling and efficiency of analysis, the relevant question is what minimum number of cores from a single tree is required to represent

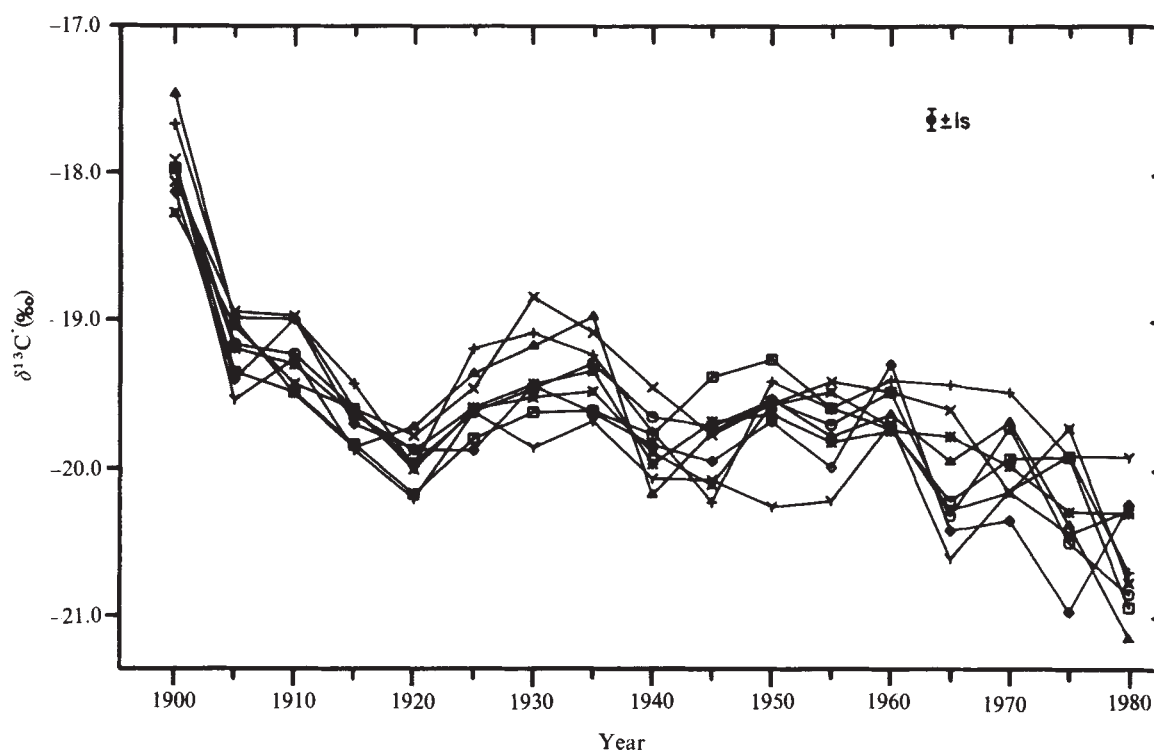


Fig. 1 The $\delta^{13}\text{C}$ trends of 5-yr ring groups (values plotted at the beginning of each 5-yr interval) from eight radii of a *P. edulis* tree sampled south of Flagstaff, Arizona. *, The $\delta^{13}\text{C}$ trend from an adjacent cross-section whose ring-groups were collected from the full circumference.

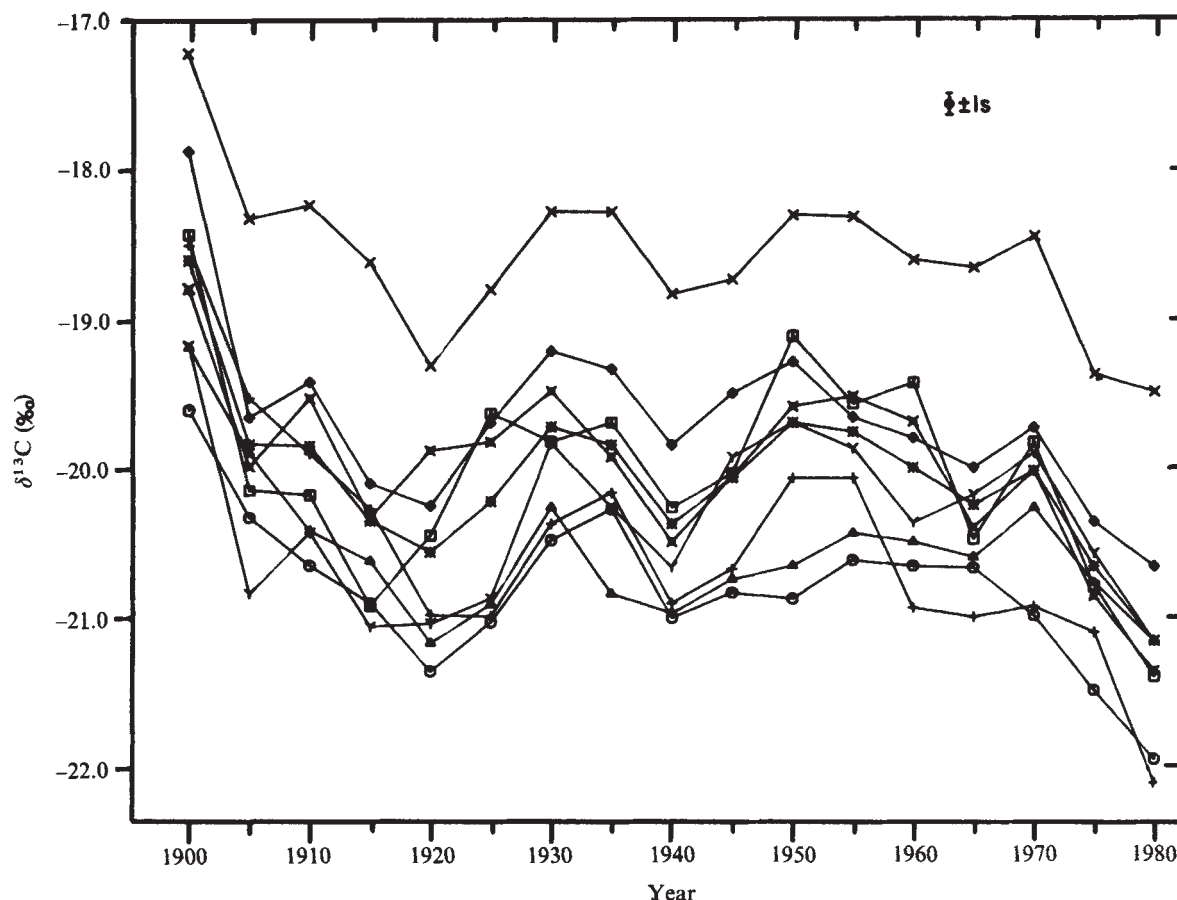


Fig. 2 The $\delta^{13}\text{C}$ trends from eight trees at the site where the tree in Fig. 1 was collected. Each tree is represented with four cores whose 5-yr ring groups were pooled before analysis. *, The eight-tree mean.

accurately the $\delta^{13}\text{C}$ of cellulose fixed in a tree-ring sequence, although the answer may actually differ among species and local environments.

The *P. edulis* cross-sections we used to help resolve this question for one environment had nearly circular, concentric rings. Furthermore, the ring series had only 1.5% missing rings over the growth period 1795–1981. Figure 1 shows the comparison between the $\delta^{13}\text{C}$ trends of the eight radial slices (simulating cores) and 'whole-ring' trend derived from full-circumference analysis of the adjacent cross-section. The $\delta^{13}\text{C}$ differences among radii at any interval range from ~0.5 to 1.2%.

An assessment of the minimum number of cores needed must involve a compromise between effort involved and proximity to the true value. To evaluate this trade-off, we compared combinations of cores with the full-circumference $\delta^{13}\text{C}$ results taken as the true value, using two tests. The Pearson correlation coefficient (r) measures the strength of the linear relationship between mean data of a given core combination and that of the cross-section, and the sum of the squares of the differences (SS) between the mean core combination and the true trend is a measure of the absolute $\delta^{13}\text{C}$ differences between the curves.

These tests were performed on selected combinations of 1–8 radii. Sampling of a given number of cores from a tree in the field would normally be done equidistantly about the tree circumference, and it is these combinations which were examined. For the eight radii of this study at 45° intervals, equidistant combinations of 2, 4 and 8 cores exist. For combinations of three, five, six and seven cores, however, equidistance could only be approximated with this set of cores. The lowest r found was 0.84 for a single radius (west) versus the true values, yet it is actually significant at 99%. However, close examination of such $\delta^{13}\text{C}$ trends with relatively low r values reveals significant differences between some of the short-term variations of the core trend and the true trend. This would limit reconstruction

of fine-scale features with single cores. Both parameters improve greatly as the number of cores increases from one to four ($\bar{r}$ increases from 0.89 to 0.96 and $\bar{SS}$ decreases from 1.54 to 0.47), but beyond four cores the improvement tails off. Two cores show a marked improvement over single cores in that not only are the correlation coefficients of each two core combination >0.90 , but the largest SS values are only slightly >1 . SS values of <1 indicate the mean deviation between core combination and true trend at each interval is $<0.25\%$, close to a non-statistical difference in light of analytical precision.

We performed this statistical analysis using (1) unweighted $\delta^{13}\text{C}$ means of the core combinations, and (2) $\delta^{13}\text{C}$ means of the core combinations weighted for the respective 5-yr ring thickness contribution of each of the selected cores, and found the results to be nearly identical, despite two- to threefold differences in ring thickness among radii for a given interval. This implies that, in application, cores may be pooled before analysis (equivalent to weighting the mean) and the results will not be distinguishable from the much more involved process of analysing each core separately and calculating a mean (equivalent to the unweighted mean). We had also tested the eight possible combinations of four cores from the same side of the tree and found they show an appreciably higher SS ($\bar{SS}=0.62$, range 0.42–0.74) than those taken from opposite directions ($\bar{SS}=0.35$, range 0.19–0.51), which suggests that orthogonal cores yield more representative samples. Based on these results we are now sampling four cores per tree wherever possible, because they represent short-term variations well and they offer an extra margin of safety over two cores.

Adequate representation of a $\delta^{13}\text{C}$ trend from a given locality requires a knowledge of the $\delta^{13}\text{C}$ variability among individuals. Very few studies have examined intra-site variation of $\delta^{13}\text{C}$ in tree rings, with results in the general range of 1–2% (refs 3, 6, 16, 18, 19). Unfortunately, single cores were generally taken as

representative of each tree in these studies. Our study compares $\delta^{13}\text{C}$ trends in eight trees from a single site sampled with four orthogonal cores per tree. These trees were within an area of ~ 2 ha of an open pinyon-juniper woodland with typical distances of 5–10 m between individuals. The goal was not to sample eight identical trees, but to sample eight trees having no obvious characteristics which would disqualify them in routine sampling. The physical setting was uniform in that the terrane was nearly flat or sloping slightly WNW, and the soil was developed on basalt bedrock. We selected pinyons of apparent good health although each had a different canopy configuration. The heights of the sampled trees ranged from 5 to 7 m, the diameters (1 m above ground) were ~ 20 –45 cm, and the ages varied from ~ 130 to 300 yr. For each tree, 5-yr ring groups from the four cores were combined before chemical preparation and analysis.

Figure 2 shows the $\delta^{13}\text{C}$ trends from these eight trees and the mean trend. The $\delta^{13}\text{C}$ values for each time interval range over 2.5‰ among the trees. If not for the one tree which shows distinctly heavy isotopic values, the range would be more of the order of 1.5‰. We found no relationship between the absolute $\delta^{13}\text{C}$ values of the trends and the respective ages, heights, diameters, crown shapes, or proximity to neighbours of the trees.

As in the case of the radial results, it is important to determine how many trees would be required to give an accurate representation of both the $\delta^{13}\text{C}$ trend and absolute $\delta^{13}\text{C}$ values registered in the trees of the site. No absolute reference value exists for comparison, so we have used the mean of the eight trees as the true value. The unweighted mean seems logical as there is no *a priori* reason to suspect that any one tree is a more reliable recorder than the next. It was also necessary to examine many more combinations of individuals in the site test than in the circumferential test in order to simulate all field collection possibilities.

The δ values of each tree were first weighted for their respective mean 5-yr group ring widths, which typically varied 3-to 4-fold among trees, before calculating the mean trend of each combination of trees. This is equivalent to pooling trees before analysis. The pattern of r and SS results is similar to those from the intra-tree analysis, although due to the wider range in $\delta^{13}\text{C}$ values among the trees, the SS values are higher. As in the intra-tree analysis, it appears possible for a single tree to be highly representative of the site (high r , low SS), but in the mean ($\bar{r} = 0.91$, $\bar{SS} = 8.51$) and extreme, individual trees will be much less representative than larger combinations. Sampling four trees per site yields highly significant r values ($\bar{r} = 0.98$) and low SS ($\bar{SS} = 0.80$), making it a good choice for practical application. The diminishing returns of larger samples may not justify the added sampling expense and energy. We are now collecting cores from six to eight trees per site and choosing four trees with the best dating, fewest missing rings, fewest obscured rings, and so on, for a pooled sample to represent the site $\delta^{13}\text{C}$ trend. These pooled test results show no significant differences from a similar compilation comparing unweighted means of the selected trees with the true value trend. Furthermore, these results differ little from those of similar tests where we used a weighted (by ring thickness) mean trend of the eight trees as the 'true' trend.

In addition to tree-ring isotope studies for reconstruction of climate and past atmospheric carbon dioxide, these results may be useful in designing and interpreting growth-chamber experiments where environmental effects on $\delta^{13}\text{C}$ are tested, or to food-web studies where stable isotopic compositions of organic matter are used to determine carbon sources and contributions. However, we must warn against extrapolating circumferential relationships developed from one pinyon pine to all species and environments. In view of the factors influencing $\delta^{13}\text{C}$ in plants described by Francey and Farquhar⁹, microenvironmental factors such as shading may enhance circumferential variability. Perhaps the $\delta^{13}\text{C}$ differences among radii of any tree may ultimately be used to quantify the microenvironmental differences in the crown which produce the circumferential variability.

Within a site, the range of 2–3‰ among pinyon pine is actually quite similar to other studies with other species, despite their being sampled with single cores and representing site environments often quite distinct from the semi-arid sites in which pinyon commonly occur. This range of $\delta^{13}\text{C}$ values at a site must be considered in sampling, particularly when attempting to relate the differences in absolute $\delta^{13}\text{C}$ values among sites to the different climates of the respective sites. We had found such a $\delta^{13}\text{C}$ -climate relationship in a study with juniper⁵, but we had represented the $\delta^{13}\text{C}$ values of each site with only one tree.

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Correlation of marine and continental glacial and interglacial events, Arctic Ocean and Banks Island

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Correlation of Pleistocene glacial and interglacial sediments deposited on land with sediments deposited synchronously in the ocean basins is rarely accomplished^{1–3}. A significant exception is the correlation of continental shelf Bay of Biscay interglacial deposits with interglacial sediments from north-east France using $\delta^{18}\text{O}$ and pollen⁴. In the Arctic Ocean, the definition of a partial magnetic stratigraphy for a Pleistocene sequence on Banks Island, western Arctic Archipelago⁵, permits correlation with magnetically defined marine sediment of the Chukchi–Alpha Ridge^{6–8} (Fig. 1). We report here the details of this first Arctic Ocean glacial-marine to continental-glacial deposit correlation, and its climatic interpretation. Arctic Ocean sediment texture is climatically controlled and fine-grained textured sediment was deposited in the ocean at the same time as interglacial deposits formed on the adjacent land. The occurrence of coarse-grained Arctic Ocean sediment on the other hand is synchronous with glacial advances on land and also represents periods of deglaciation.

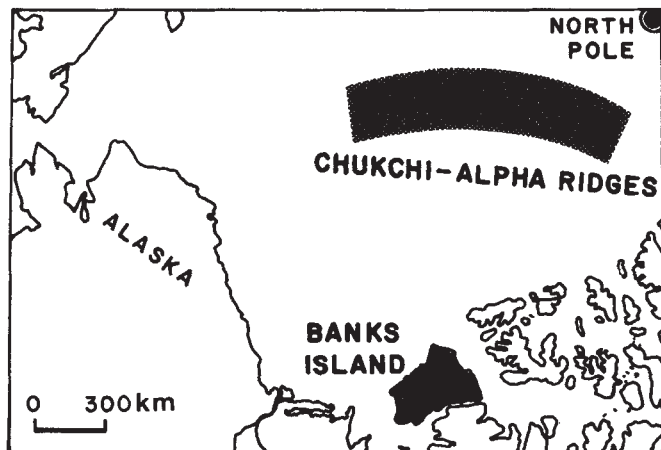


Fig. 1 Location of Banks Island and the area of glacial-marine stratigraphy developed for the Chukchi-Alpha Ridges.

Palaeomagnetic interpretations of Arctic Ocean sediment have been based primarily on polarity signatures (+ or -) published without supporting inclination, intensity or virtual geomagnetic pole (VGP) latitude data^{6,9}. However, these data have been discussed recently⁸, with inclination data for a representative Arctic core (and similar data for the Banks Island record) (Fig. 2) confirming the validity of previous interpretations. Demagnetization and VGP data have been published for Banks Island⁵. Supporting data on demagnetization, inclination and intensity of the marine cores not considered here, will be published elsewhere¹⁰.

The Brunhes-Matuyama magnetic boundary, interpreted to be 730 kyr, has been recognized in numerous sediment cores taken from ice-island T-3. The reversal from normal to reversed polarity occurs consistently in stratigraphical unit K⁷, which is widely recognized throughout the Amerasian Basin of the Central Arctic Ocean and also reliably correlates with sediment of the Lomonosov Ridge¹¹. Unit K is a dark yellowish-brown foraminifera-rich, silty lutite that ranges in thickness from 14 to 53 cm, is intensively burrowed and is finer-grained than adjacent units. It has been interpreted as representing an interval of reduced glacial ice influx in the Arctic (that is, the glacial ice minimum).

Sediments on Banks Island provide the best record of Pleistocene events in the western Canadian Arctic Archipelago. Three continental glaciations (Banks, Thomsen and Amundsen) as well as preglacial and intervening interglacial periods (Morgan Bluffs and Cape Collinson) have been documented^{12,13} (Fig. 3).

A preliminary palaeomagnetic investigation⁵ of the Duck Hawk Bluffs of southwestern Banks Island, where all recognized glacial and nonglacial events are recorded¹⁴, indicates that the

preglacial sediments, and the glacial and marine deposits laid down during the oldest and most extensive Banks Glaciation, and during part of the Morgan Bluffs Interglacial, are probably of Matuyama age (>730 kyr) (Fig. 3). Younger terrestrial deposits associated with the Morgan Bluffs and Cape Collinson interglacials, and the glacial and marine deposits associated with the Thomsen and Amundsen glaciations, are normally magnetized and probably of Brunhes age (<730 kyr). The Banks Glaciation is the only one in which continental ice overrode most of Banks Island and probably large areas of other western Arctic Islands, extending some distance in the ocean. During the younger glacial stages the ice filled some of the inter-island channels and impinged on coastal areas but may have not extended very far into the ocean.

Figure 3 is a tentative correlation of the glacial and interglacial deposits of Banks Island with the lithostratigraphical units recognized in the central Arctic Ocean. The Morgan Bluffs Formation includes terrestrial interglacial deposits and unit K represents a time of reduced glacial ice activity in the ocean. The younger Thomsen and Amundsen glaciations, the Cape Collinson Interglaciation, and Holocene deposits from Banks Island correlate with the upper part of unit K, and units L and M, which are glacial-marine units that include alternating coarse- and fine-textured sediment; these represent alternating intensive and less intensive ice rafting.

The older Banks Glaciation glacial and associated marine deposits, represented by the Duck Hawk Bluffs Formation (Fig. 2), record the strongest advance of a continental ice sheet in the western Canadian Arctic Archipelago and are probably correlative with unit J in the oceanic record. Unit J is one of the coarsest grained units of the late Cenozoic stratigraphy and commonly shows a large coarse peak on graphs of texture (Fig. 4). Because the Banks Glaciation is the strongest glacial advance recognized in the western Canadian Arctic, it is reasonable to expect that the ocean record of this event was a very pronounced increase in coarse sediment. In detail, unit J (Fig. 4) commonly has two prominent coarse zones separated by a finer-grained interval. This suggests that if a correlation with the Banks Glaciation is real, the Banks interval may have included more than one surge of ice, a fact which should be recorded on southern Banks Island by the presence of multiple stacked and distinct Banks Glaciation tills¹³. Because terrestrial glacial and interglacial deposits could experience more erosion than their equivalents in the ocean, the marine record should be more complete. It has also been suggested that unit J can be correlated with oxygen isotope glacial stage 22 (ref. 7).

The recognition of the Brunhes-Matuyama magnetic signature in Pleistocene sediments of Banks Island permits the first correlation of Canadian continental glacial and interglacial deposits with Arctic Ocean sediment. The Morgan Bluffs Formation is interpreted to be an interglacial deposit and its correlative, unit K, is a silty lutite that has been interpreted to have been deposited during time of reduced continental glacial ice activity³.

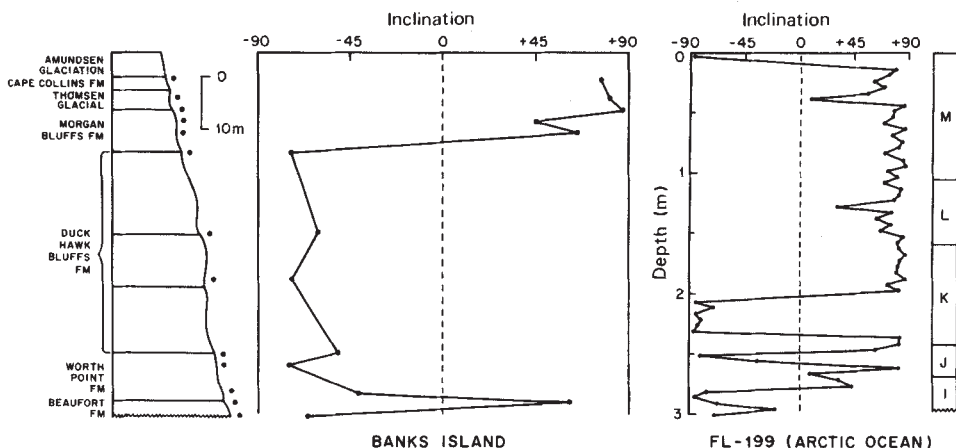


Fig. 2 Magnetic inclination plots for a representative Central Arctic Ocean core compared with a similar plot for Banks Island; the Arctic plot is representative of cores^{8,10}, and the Banks Island data are based on multiple samples⁵. Note that Banks Glaciation is represented by Duck Hawk Bluffs Formation.

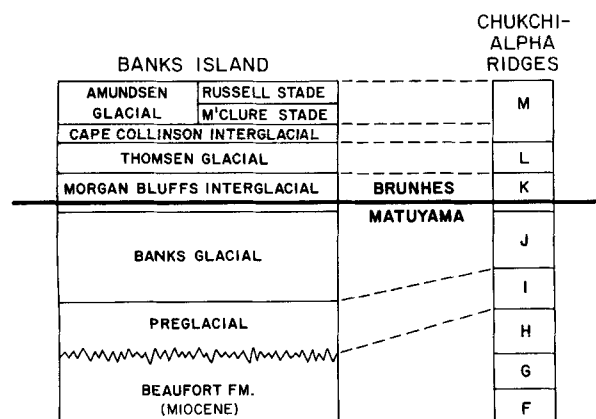


Fig. 3 Correlation of the Banks Island terrestrial deposits^{8,9} and glacial-marine stratigraphy of the central Arctic Ocean⁷. The Brunhes normal and Matuyama reversed magnetic intervals in terrestrial and marine sequences are indicated. Marine unit K has previously been interpreted to be of interglacial marine origin⁷.

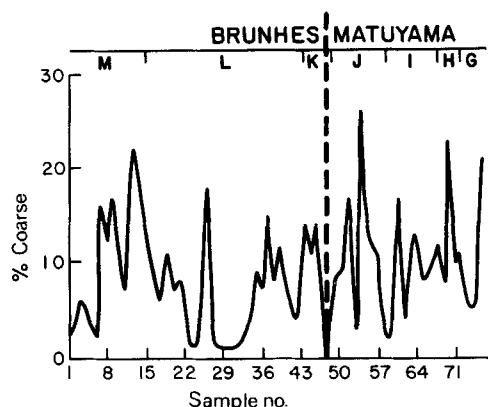


Fig. 4 Per cent coarse sediment (>62 μm) in Arctic Ocean core FI-196, at 80°33' N, 171°34' W, from 3,327 m depth. '1', is the core top with samples at 1-cm intervals. The Brunhes-Matuyama magnetic boundary (730 kyr) is indicated as is M, L, K, J, I, H, and G stratigraphy for the core⁷. Coarse peak in J probably correlates with Banks Glacial (Duck Hawk Bluffs Formation, Figs 2, 3).

The other stratigraphical units identified in the Arctic Ocean and differentiated by texture provide a record of glacial and interglacial events that can be correlated with the terrestrial deposits. This apparent confirmation of the significance of oceanic glacial-marine textural relationships with continental glacial and interglacial sediments provides a powerful tool for marine-terrestrial-glacial correlations. This Arctic terrestrial-marine correlation should permit significant palaeoclimatic interpretations that are based on sediment texture.

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Patterns of junctional communication in the early amphibian embryo

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It has long been recognized that cells in early embryos can communicate with each other via a direct cell-to-cell pathway, probably mediated by gap junctions. Low electrical resistance pathways, detected electrophysiologically, have been identified in all species examined so far. However, studies in various embryos on the transfer of molecules larger than small ions (for example, fluorescent dyes in the molecular weight range 350-500) have given conflicting results¹⁻⁵. In all these studies the ability to transfer dyes from cell to cell was determined without reference to the position of the injected cell in the embryo. In the experiments reported here, cell-cell transfer of the fluorescent dye, Lucifer yellow⁶ (molecular weight (M_r) 450) was re-examined in the early *Xenopus laevis* embryo by injecting the dye into identified cells, as the position of the injected cell within the embryo may be important. At the 32-cell stage, we found that dye transfer often occurred between animal pole blastomeres which were not sisters, as well as between sister cells, and also that Lucifer yellow was indeed transferred via gap junctions. The cell-cell transfer was not uniform within the animal pole; transfer was maximal near the dorsal side and minimal at the ventral side. This pattern may reflect differences in permeability or numbers of gap junctions across the embryo, and could be related to early events in development.

Previous studies¹⁻⁵ investigating the transfer from cell to cell of fluorescent dyes in the molecular weight range 300-500 in various embryos have yielded inconsistent results. Dye transfer has been reported in *Fundulus* embryos¹, mouse embryos² and blastomeres from axolotl embryos³. Conversely, the absence of cell-cell transfer of such dyes has also been demonstrated in *Fundulus*³, and in starfish embryo⁴ and blastomeres of early *Xenopus* embryos⁵. These studies failed to identify the position of the injected cell within the embryo, and it is now clear that restriction of dye transfer can occur at the site of a developmental boundary, for example, the segmental border in the insect *Oncopeltus*⁷. This has also been suggested by experiments in the mouse embryo² and in *Drosophila* imaginal disk⁸. Thus it is possible that transfer between cells of molecules in this molecular weight range depends on the position of the injected cell in the early embryo. This possibility has now been tested in the early *Xenopus* embryo.

All injections were made at the 32-cell stage of development. In preliminary studies, where animal pole cells were injected randomly with respect to position, Lucifer yellow was often found to pass between cells which were not sisters, frequently filling two or more other cells, but not often more than four of five. However, instances of no transfer within the observation period (up to 20 min after injection) were also observed, consistent with previous observations in this embryo⁵. In whole embryos the visual assessment of failure to transfer was sometimes complicated by pigmentation, scatter and autofluorescence. Scatter was largely eliminated by closing down the diaphragm, and scanning across cells appearing to contain dye. Frozen sections were then used to confirm the determination made on the whole embryo (see Fig. 2).

In order to eliminate complications introduced by the persistence of cytoplasmic bridges from the previous cleavage, several injections used fluorescein isothiocyanate (FITC)-conjugated dextrans, whose high molecular weight (10,000) prevents their passage through gap junctions⁹. The results showed that FITC-dextrans were often present in two cells which were daughters of the previous cleavage (that is, sisters at the 32-cell stage) but never in more than these two cells. As Lucifer yellow frequently filled cells that were not sisters, it was assumed to transfer via gap junctions. The timing of the disappearance of

Fig. 1 Map of the *Xenopus* embryo. **A**, The pigmentation pattern of the animal pole of the *Xenopus* egg, at the time when the lifting of the pigment at the grey crescent becomes apparent. The grey crescent is bisected by the primary cleavage axis, and is fated to become the future dorsal side of the embryo, while the opposite side will be ventral. Eggs were obtained by inducing pairs of *Xenopus laevis* to lay by injection of chorionic gonadotropin. **B**, The nomenclature of the cells used in these experiments in a 32-cell embryo, viewed from the animal pole. Embryos at the 32-cell stage, with regular cleavage pattern and identifiable grey crescent were mechanically dejellied and placed in a wax bath filled with half-strength Ringer's solution (60 mM NaCl, 1.25 mM KCl, 4 mM CaCl₂, 1 mM Tris, pH 7.4) in their normal orientation with the animal pole upwards. Sixteen radical cells were then visible, with pairs of sister cells lying in the same segment, and position identifiable with respect to the grey crescent. **C**, The predominant dye movements in the 32-cell embryo, labelled with the direction and number of instances of transfer. Because of the unknown duration of cytoplasmic bridges between sister cells, dye movements are shown between segments of the animal pole, each consisting of two sisters of the previous cleavage. Note that transfer was bidirectional across all segments except across the primary cleavage axis at the ventral end. The high incidence of transfer across the axis at the dorsal side, from segment *d* to *e* and vice versa, emphasized this asymmetry.

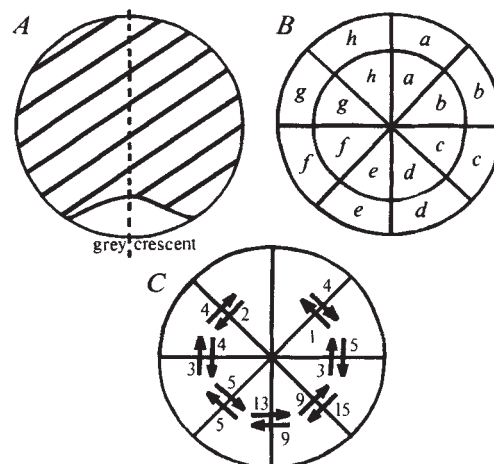
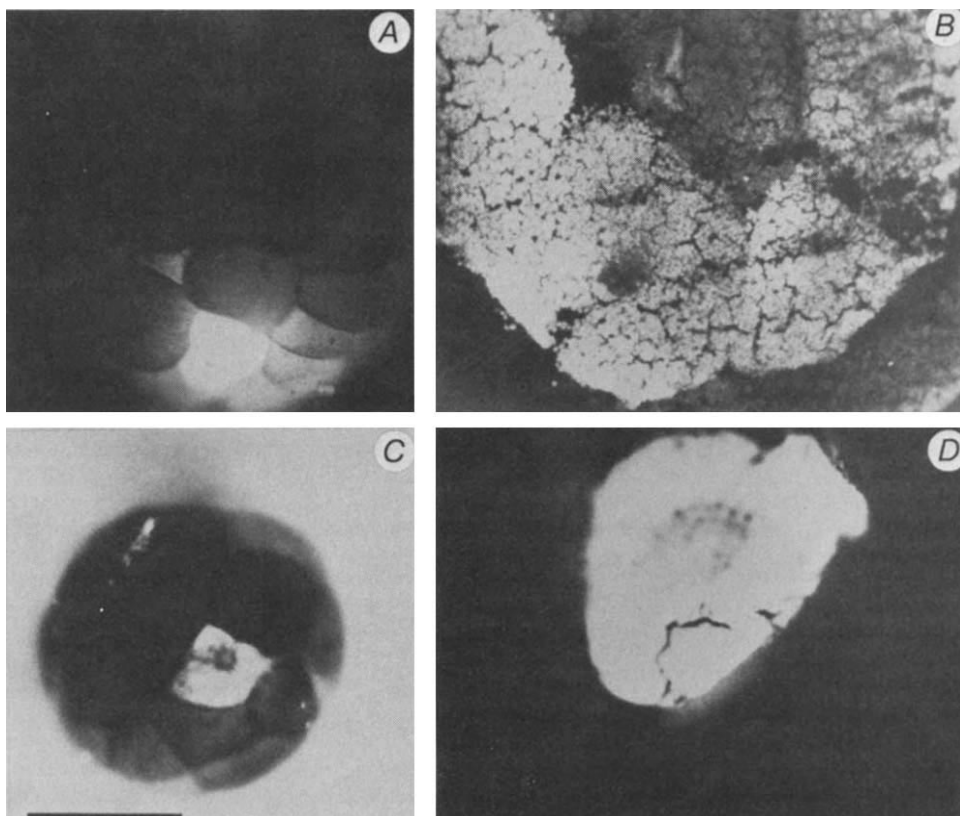


Fig. 2 Photographs of Lucifer yellow-injected embryos. **A**, A whole embryo injected with Lucifer yellow into cell *d* (inner ring) at the 32-cell stage. The embryo has begun to cleave to 64 cells, and there is extensive transfer into several other cells. Scale bar, 500 μ m. **B**, A frozen section of an embryo with dye injected into cell *d*. The injected cell is the largest in the field of view with a prominent nucleus, and has transferred dye in the lateral direction to three other cells. Scale bar, 200 μ m. **C**, A whole embryo with dye injected into cell *a* (inner ring) at the 32-cell stage with no transfer apparent. Scale bar, 600 μ m. **D**, A frozen section of an embryo with dye injected into cell *a*, with sharp restriction of dye to the injected cell. Scale bar, 200 μ m.



Methods: For Lucifer yellow injection, microelectrodes were filled with 2% Lucifer yellow, and backfilled with 1% LiCl (resistance 20 M Ω). After impalement and sealing, indicated by a good resting potential (20 mV), rectangular hyperpolarizing pulses (100 nA, 30 ms duration, 10 Hz) were used to iontophorese dye into cells, using a standard injection time of 5 min. To inject 4% FITC-dextran (Sigma) I used a hand syringe or pressure injection unit (Picospritzer II). Immediately after injection, embryos were examined by fluorescence microscopy, and the pattern of dye spread out from the injected cell was noted diagrammatically. The injected cell was often slightly swollen, but this had no deleterious effects, as injected embryos could be grown into normal tadpoles in tap-water. Some embryos were fixed in 4% formaldehyde for frozen sectioning (1 h fixation followed by freezing in isopentane and sectioning at 20 μ m on a Bright 5030 microtome) or plastic sectioning (4–5 h fixation followed by dehydration in alcohols, overnight infiltration and embedding in methacrylate resin, then sectioning at 10 μ m on a Du Pont Sorvall UBA microtome).

cytoplasmic bridges varied from embryo to embryo; all cases of transfer of Lucifer yellow into the sister cell only were therefore scored as instances of failure to transfer.

To test whether the non-uniform transfer of dye between cells was related to the position of the cell within the animal pole, injections were made into cells whose position had been reliably identified with respect to the primary cleavage axis and the grey crescent. Figure 1A and B shows the nomenclature used. Sixteen injections were made into cells at each position, so that 256 embryos were injected in all. Photographs of whole embryos and sections showing examples of presence and absence of transfer are shown in Fig. 2.

The pattern which emerged was that cells near the grey crescent transferred dye in a majority of cases, but those distant from it transferred little dye. The data from all these experiments are given in Fig. 3, as plots of the number of embryos (out of a total of 16) for each identified cell, which showed no transfer,

transfer to sister cell only, or transfer to one or more other cells, not including the sister. For the inner ring, cells *c*, *d* and *e*, at the grey crescent side (future dorsal), clearly exhibited the best transfer. By contrast, cells *a* and *h* (at the future ventral side) rarely transferred dye into their lateral neighbours. The pattern of transfer was shifted slightly to the right of the primary cleavage axis at the grey crescent end. A similar pattern was seen for the outer ring of cells, although the total amount of transfer was substantially greater. Table 1 summarizes the data from these experiments, giving the percentage of observed cases of transfer and no transfer for each cell. From this it is clear that even cells which frequently transferred dye, for example cell *c*, could sometimes fail to transfer, just as cells which seldom transferred dye occasionally showed the opposite behaviour. There is no evidence for corresponding differences in electrical coupling across the embryo, though accurate quantitative measurements of the extent of electrical coupling are difficult to obtain. Using

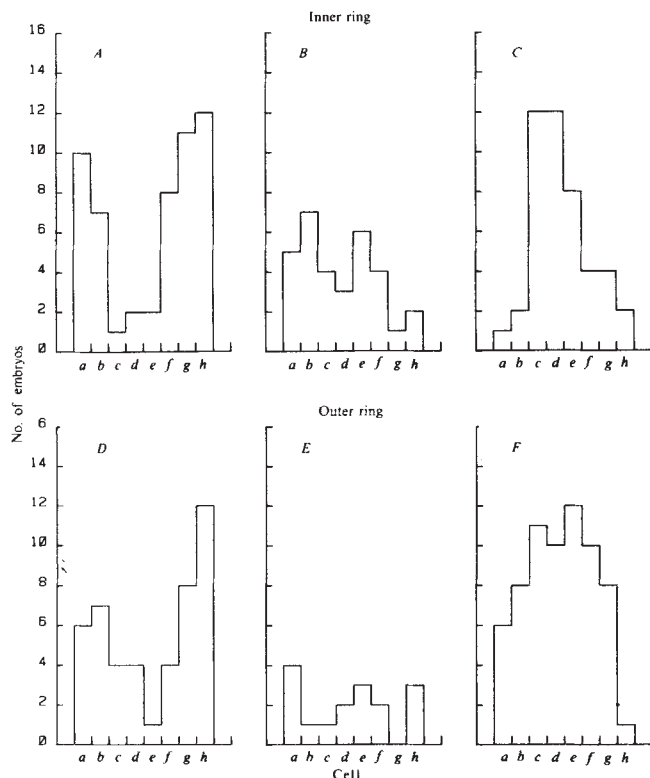


Fig. 3 Graphs showing distribution of Lucifer yellow transfer in the 32-cell embryo. Plots A and D show the number of instances of no transfer from cells in the inner and outer ring respectively. B and E show incidence of transfer to sister cell only, and C and F incidence of transfer to one or more other cells not including the sister cell. The irregularity of graphs B and E reflects the finding that transfer between sister cells appears to be random with respect to position. Graphs A and D show that the least transfer occurred at the non-grey crescent side of the embryo, while the predominance of transfer at the grey crescent is evident from graphs C and F, especially for cells c and d in the inner ring, and cell e in the outer ring.

the χ^2 test, statistical comparison of the observed patterns of transfer on the assumption that there was an equal probability of observing transfer throughout the embryo showed that the chance of these patterns occurring at random is $<1\%$.

Examination of the qualitative nature of the pattern of dye transfer showed preferences in the direction of transfer from individual cells. The major dye movements in the 32-cell embryo are mapped in Fig. 1C. As noted above, dye transfer occurred predominantly at the grey crescent side of the embryo, especially between segments d and e. A striking finding was the presence of bidirectional transfer between all segments except at the ventral side between a and h, where no instance of transfer was observed.

The data presented here suggest that there were pronounced asymmetries in the degree and direction of spread of dye from cells of the early *Xenopus* embryo. Differences in number or permeability of gap junctions could be responsible for the gradient of transfer from the dorsal to ventral side, though the factors which govern direct cell-cell communication in embryonic systems are as yet unknown. The more interesting question raised by these findings, however, is whether this phenomenon has developmental implications; evidence of stage-dependent changes in the pattern of dye transfer would support this. Further experiments on the early *Xenopus* embryo have shown a progressive restriction of dye transfer from the 16-cell through to the 32- and 64-cell stages, followed by an increase at the early blastula stage. These results will be elaborated elsewhere, together with data from injections using probes of different sizes. Manipulations which disrupt the pattern of cell to cell communication, and produce a parallel disruption in development, would

Table 1 Dye transfer between identified animal pole cells at the 32-cell stage

	Instances of no transfer or transfer to sister cell only % (n)	Instances of transfer to one or more other cells, not sister cells % (n)
Inner ring		
Cell a	94 (15)	6 (1)
b	87.5 (14)	12.5 (2)
c	31 (5)	69 (11)
d	31 (5)	69 (11)
e	50 (8)	50 (8)
f	75 (12)	25 (4)
g	75 (12)	25 (4)
h	87.5 (14)	12.5 (2)
Outer ring		
a	62.5 (10)	37.5 (6)
b	50 (8)	50 (8)
c	31 (5)	69 (11)
d	37.5 (6)	62.5 (10)
e	25 (4)	75 (12)
f	37.5 (6)	62.5 (10)
g	50 (8)	50 (8)
h	94 (15)	6 (1)

The numbers and percentages of transfer and cases of no transfer observed for each cell. Instances of transfer to the sister cell only were regarded as no transfer, as sister cell transfer depended on the time in the cleavage cycle when the injection was made. The observed distribution of transfer from all cells examined was compared with a distribution in which the probability of transfer from all cells was taken to be 50%, by the χ^2 test. The observed distribution was shown to be significantly different ($0.01 > P > 0.005$). For a comparison between the observed results and a distribution in which the probability of transfer is 100%, $0.005 > P$. n, Number of embryos.

also provide persuasive evidence for a developmental link. One such manipulation is described elsewhere in this issue¹⁰.

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Transient expression of a surface antigen on a small subset of neurones during embryonic development

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During development, neurones find and interconnect with their targets in a remarkably precise way. The unfolding of neuronal specificity involves a series of highly specific recognition events which are likely to be coordinated by the spatial and temporal expression of many different surface molecules. At early stages of development, neuronal recognition occurs most dramatically at the tips of growing axons, at growth cones and their filopodia^{1,2}. Previous studies on the grasshopper embryo suggest that specific filopodial contacts lead to the stereotyped patterns of selective

axonal fasciculation; these results support the 'labelled pathways' hypothesis which predicts that the different neighbouring axon fascicles in the embryonic neuropil within filopodial grasp are differentially labelled³⁻⁸. To uncover the molecular labels on fasciculating embryonic axons, we screened 2,000 monoclonal antibodies generated against the embryonic neuroepithelium⁹. Here we describe three antibodies which reveal surface antigens whose temporal and spatial expression during embryogenesis correlate with the predictions of the model. In particular, the Mes-2 antibody recognizes an antigen which is transiently expressed on the surface of only 4 out of ~1,000 neurones in each metathoracic hemisegment during a short period of embryogenesis. The growth cones of two of these neurones fasciculate in the periphery and innervate the same target. Moreover, they transiently express the Mes-2 surface antigen while doing so.

In the 40% grasshopper embryo, the G growth cone is within filopodial grasp of ~25 different longitudinal axon fascicles (made up of ~100 different axons), and yet invariably fasciculates on the A/P fascicle; when the A/P fascicle is ablated, the G growth cone does not show a high affinity for any other fascicle⁵⁻⁸. Furthermore, extensive ultrastructural⁷ and experimental⁸ analysis demonstrates that G is able to distinguish the two P axons from the two A axons within the A/P fascicle. Given this specificity, it seems reasonable to hypothesize that each of the ~25 longitudinal axon fascicles, and perhaps subsets of axons within the fascicles, are differentially labelled.

To identify molecules whose temporal and spatial expression correlate with this prediction, we generated monoclonal antibodies against the grasshopper embryo central nervous system (CNS)⁹. In three fusions (NS-1 myeloma cells), BALB/c mice were immunized with 40% grasshopper neuroepithelium; in a fourth fusion (from which monoclonal antibody Mes-2 was generated), mice were first immunized with whole *Drosophila* embryos and boosted with 40% grasshopper neuroepithelium. Approximately 2,000 wells were screened on lightly fixed whole-mount embryos.

The Mes-3 and Mes-4 monoclonal antibodies stain only one longitudinal axon bundle (that pioneered by the MP1 and dMP2 axons) out of ~25 different fascicles in each hemisegment in the 40% embryo (Fig. 1d); the antigens recognized by these antibodies are expressed from the beginning of axonogenesis to the end of embryogenesis. This demonstrates that an individual fascicle is antigenically distinct from all other fascicles within filopodial grasp at the time growth cones are choosing between them. The Mes-3 and Mes-4 antigens (as well as the Mes-2 antigen described below) are also expressed on the surface of the muscle pioneers, large mesodermal cells which provide guidance cues for motoneurone growth cones¹⁰. The finding that all three antibodies recognize the surfaces of the muscle pioneers is striking, but its significance is unclear.

The Mes-2 antigen is transiently expressed on only 4 out of ~1,000 neurones in each metathoracic (T3) hemisegment (Fig. 1a, b) during a short period of development (Table 1). The four neurones comprise two interneurons (IN X and IN Y; little is known about their central axon pathways), and the two excitatory motoneurons that innervate the extensor tibiae (ETi) muscle in the leg—FETi (F in Fig. 1a, b; from neuroblast 4-4) and SETi (S in Fig. 1b, c; from neuroblast 2-3). Staining of the cells with Mes-2 antibody in either highly dissected living preparations or fixed but unpermeabilized preparations (cytoplasmic antigens require detergent treatment in these whole-mount preparations) indicates that the Mes-2 antigen is on the cell surface.

The growth cones of FETi and SETi follow the same final axonal pathway and innervate the same muscle. Of the ~100 motoneurons in each T3 hemisegment whose growth cones innervate muscles in the body wall or limb bud, only FETi and SETi express the Mes-2 antigen. Although these two motoneurons arise from different neuroblasts, they alone amongst their two families share a final axonal pathway and target, and express Mes-2. Two other neurones also innervate this muscle later in development: they are the CI inhibitory

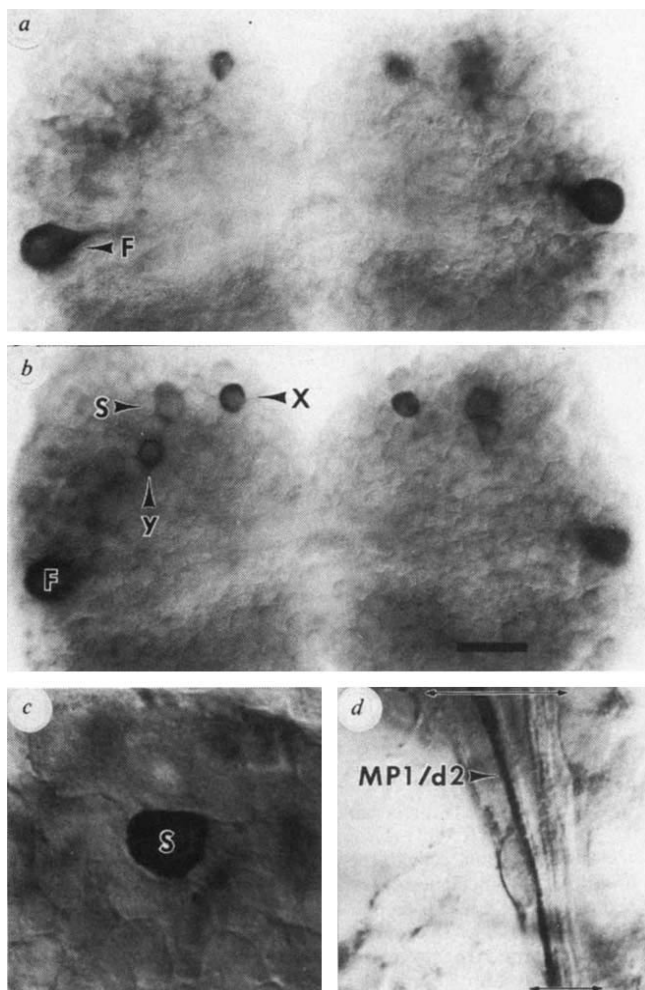


Fig. 1 Binding of Mes-2 (a-c) and Mes-3 (d) monoclonal antibodies to small subsets of neurones in the grasshopper embryo. a, b, Two different focal planes of a 60% metathoracic (T3) ganglion showing four cell bodies (out of 1,000 on each side) labelled by Mes-2 antibody: F, FETi, fast extensor tibiae motoneurone; S, SETi, slow extensor tibiae motoneurone; X, interneurone X; Y, interneurone Y. The Mes-2 recognizes a surface antigen that is transiently expressed on the axons and cell bodies of these four neurones. c, Transient expression of Mes-2 antigen (see Table 1). At 55% of development shown here, SETi expresses the antigen at a high level, whereas by 60% (b), SETi's expression is already declining, in contrast to that of FETi and interneurone X. d, The MP1/dMP2 fascicle is specifically labelled by the Mes-3 antibody. Only one out of ~20 axon bundles is stained in the longitudinal connective at 38% of development. Lines with arrows shown at the top and bottom of the figure mark the medial (left) and lateral (right) extent of the longitudinal axon fascicles as shown here on the right side of a single segment. Horseradish peroxidase (HRP) immunohistochemistry was performed by fixing embryos in 2% paraformaldehyde for 1/2 h, followed by incubation with antibody supernatant and subsequent protocol for the biotin/avidin HRP system (Vectastain ABC kit, Vector Laboratories). Although the Mes-2 and Mes-3 antibodies bind to surface antigens in living embryos, in all photographs shown here, 0.2% saponin was added for better visualization. Such detergent treatment reveals that the Mes-2 antigen is also within the cytoplasm in the cell bodies of the four neurones. Calibration bar: a, b, 40 μ m; c, d, 15 μ m.

Table 1 Transient expression of Mes-2 surface antigen

Cell	Staining at (% embryonic development):							
	40	45	50	55	60	65	70	80
SETi	—	—	++	++	+	+	—	—
FETi	—	—	++	++	++	—	—	—
In X	—	—	+	+	++	++	++	++
In Y	—	—	—	—	+	++	+	+

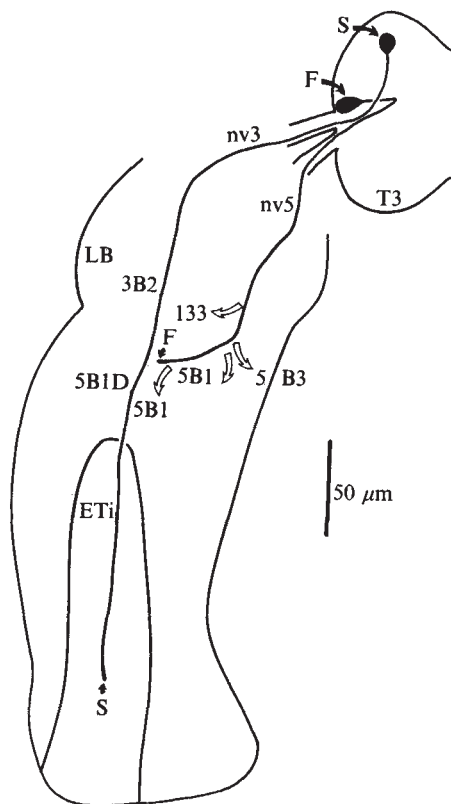


Fig. 2 Camera lucida drawing of the axons of the SETi and FETi motoneurons at 48.5% of development. At this stage, both FETi and SETi have just begun to express the Mes-2 antigen on their surface, as indicated by double labelling experiments using the Mes-2 monoclonal antibody and intracellular dye injections of Lucifer yellow followed by HRP immunocytochemistry with the anti-Lucifer yellow antibody¹². At this stage, FETi's growth cone is just about to contact SETi's axon in the femur of the limb bud (LB). The cell bodies of both motoneurons are in the T3 ganglion and SETi's growth cone at this stage is already extending across the extensor tibiae (ETi) muscle pioneers¹⁰. On its way to its target, FETi's growth cone came within 2 μ m of SETi's axon as it left the CNS; at that stage, neither cell expressed the Mes-2 antigen. On its way out as nerve 5, FETi's growth cone extended past the muscle pioneer for coxal muscle 133a, where the growth cones of three motoneurons branch off. It then reached a pathway trifurcation in the limb bud, in which it extended along nerve 5B1 instead of nerves 5B3 (followed by flexor motoneurons) or nerve 5B2 (followed by tarsal motoneurons). It then extended off from nerve 5B1 towards SETi's axon in nerve 3B2 (which becomes 5B1D). After the time shown in this figure, FETi fasciculates on SETi's axon and extends distally towards the ETi muscle. For implications, see text. Unfortunately, technical difficulties of antibody access in the 48.5% limb bud prevented us from using antibodies to block function in the living embryo.

motoneurone which also innervates several other muscles, and the DUMETi modulatory neurone which innervates other targets as well.

The Mes-2 antigen is not expressed on either FETi or SETi at the time their growth cones are extending along different central and peripheral axonal pathways. SETi's growth cone pioneers nerve 3 (ref. 11) and extends distally into the femur of the metathoracic limb bud where it extends along the ETi muscle pioneers¹⁰. At about this time, the FETi growth cone reaches the exit point of the CNS where several axon fascicles diverge as they extend distally as peripheral nerves. Although FETi's growth cone is within short filopodial grasp (2 μ m) of SETi's axon, it does not fasciculate with SETi but instead extends out nerve 5 (Fig. 2). At this stage (~40%), neither cell expresses the Mes-2. FETi's growth cone extends out into the limb bud and then makes several other pathway choices.

At 49% of development, FETi's growth cone once again comes into contact with SETi's axon in the femur of the limb bud, and

now fasciculates with SETi's axon, following it distally to the ETi muscle pioneers; at this stage of development it is the only axon to do this. Clearly, one or both of the cells has changed since their previous encounter over 24 h earlier.

Double labelling of the neurones with the Mes-2 antibody and intracellular injections of the dye Lucifer yellow reveal that expression of Mes-2 on the processes and cell bodies of both cells begins at 48.5%, just before FETi's growth cone reaches SETi's axon. Their expression of Mes-2 is transient (Table 1; compare S in Fig. 1c at 55% and b at 60%). After reaching and innervating their common muscle, expression of the Mes-2 antigen decreases dramatically and eventually disappears, and does not reappear during larval and adult life.

The data suggest that the Mes-2 antigen may be involved in the selective fasciculation of FETi's growth cone onto SETi's axon. Only when both cells express the antigen on their surface do they fasciculate. The Mes-2 antibody demonstrates (1) the remarkable specificity of a surface antigen on a very small subset of neurones (4 out of 1,000, 2 of which fasciculate in the periphery) and (2) the transient expression of a surface antigen during the narrow time window of development in which axon outgrowth and cell recognition occurs. It will thus be of interest to isolate the surface molecules recognized by such antibodies as Mes-2, Mes-3 and Mes-4, and to characterize their function during neuronal development.

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Neural cell adhesion molecules and myelin-associated glycoprotein share a common carbohydrate moiety recognized by monoclonal antibodies L2 and HNK-1

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Cell surface molecules have been implicated in cell interactions which underlie formation of the nervous system. The analysis of the functional properties of such molecules has profited from the combined use of antibodies and cell culture systems. It has been suggested that the interplay between these molecules modulates cell-to-cell interaction at critical developmental stages^{1,2}. In the mouse, N-CAM^{1,3} and L1 antigen⁴ have been shown to mediate Ca^{2+} -independent adhesion among neural cells. N-CAM plays a role in fasciculation of neurites and formation of neuromuscular

junction. L1 is apparently not involved in synaptogenesis, but in migration of granule cell neurones in the developing mouse cerebellar cortex⁵. The two antigens are distinct molecular and functional entities which act synergistically in aggregation of neuroblastoma⁶ and early postnatal cerebellar cells⁷. In view of a certain similarity in function between the two groups of molecules, it was not surprising to find that structural similarities are detectable by the monoclonal antibody L2. We show here that a carbohydrate moiety recognized by L2 and HNK-1 monoclonal antibodies, is present in mouse N-CAM and L1. The L2 epitope appears on all major neural cell types but not all N-CAM molecules express it. This heterogeneity points to a previously undetected molecular diversity which may have functional implications for modulating cell adhesion during development.

To demonstrate that L2 antibodies react with L1 antigen and mouse N-CAM Western blots were performed with the two antigens which had been immunoaffinity purified on the respective monoclonal antibody columns. In this study, monoclonal antibody BSP-2 was used to purify and test mouse N-CAM^{8,9}. L2 and HNK-1¹⁰⁻¹² antibodies recognized the 200 and 140 kilodalton (kd) constituents of L1 and the 180 and 140, but not the 120 kd band of N-CAM isolated from adult mouse brain. In contrast, the 120 kd band of N-CAM was clearly visualized by polyclonal antiserum to BSP-2/N-CAM (Fig. 1). The two antibodies reacted with additional polypeptides present in a Nonidet P40 solubilized crude membrane fraction of adult mouse brain including two bands at 160 and 100 kd and several fainter ones in higher and lower molecular weight regions. The 160 and 100 kd bands were more clearly visualized when L1 and mouse N-CAM were removed from the membrane fraction by sequential immunoaffinity chromatography and the residual polypeptides (designated 'rest L2') recovered by L2 antibody immunoaffinity chromatography (Fig. 1). Polyclonal antiserum to MAG¹³ reacted with the 100 kd component, but not with any other polypeptide of 'rest L2' (Fig. 1).

To determine whether the L2/HNK-1 epitopes resided in the carbohydrate or protein moieties, biochemical modifications of the total glycoproteins expressing the L2 epitope were carried out. All L2 positive glycoproteins were obtained from adult mouse brain by affinity chromatography with a monoclonal L2 antibody column and will be designated 'L2 antigen'. The L2/HNK-1 epitopes were found to be heat resistant (95 °C, 15 min) and destroyed by periodate treatment (1 µg antigen in 46 µl 0.07 M Na *m*-periodate, pH 5.0, 16 h, 37 °C in the dark, according to ref. 14). Also, when 'L2 antigen' was treated extensively with pronase in denaturing conditions, the carbohydrate containing fraction was isolated by gel filtration and used to compete for L2 and HNK-1 antibody binding to purified 'L2 antigen' in immunospot binding (not shown) and enzyme-linked immunosorbent assay (ELISA) test (Table 1). The results obtained by both techniques and with both antibodies were similar. (It will be shown later by immunocytological methods that the two antibodies compete with each other for binding.) While similar amounts of 'L2 antigen' competed for both antibodies, inhibition by carbohydrate fraction was less efficient and 60 times less for HNK-1 (IgM) than for L2 (IgG) antibodies. Controls for the reaction consisted of carbohydrate fraction obtained in identical conditions from fetuin and used for competition in L2 antibody binding. Furthermore, the carbohydrate fraction from 'L2 antigen' was used for competition in an unrelated antibody-antigen reaction (human haemoglobin). No inhibition of antibody binding was observed in these controls. In an alternate experiment, purified 'L2 antigen' was treated with endoglycosidase F in denaturing conditions¹⁵. The digested product was then spotted onto nitrocellulose filters which do not permit the adsorption of free carbohydrate chains. L2 and HNK-1 antibodies did not react, while polyclonal L2 antibodies prepared in rabbits against 'L2 antigen' showed maximal binding.

To determine which cell types express the L2 epitope, immunocytochemical studies were carried out on cultured cells using double immunolabelling techniques with established cell

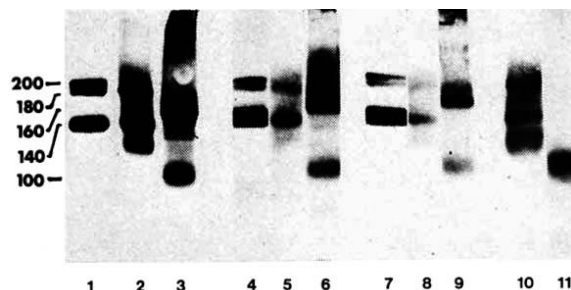


Fig. 1 Western blot analysis of immunoaffinity purified antigens L1, mouse N-CAM/BSP-2 and 'rest L2' from adult mouse brain using monoclonal antibodies L2 and HNK-1 and polyclonal antisera to BSP-2/N-CAM and MAG. Antibodies were described previously^{2,3,4,10,13}. L2 antibodies were obtained from two hybridoma clones resulting from an immunization and fusion as described for L1⁴. Another L2 antibody was obtained from a hybridoma clone resulting from an immunization with a glycoprotein enriched membrane fraction from adult mouse brain which showed neutralizing activity towards Fab fragments from antibodies to crude membranes from adult mouse cerebellum in reaggregation of dispase-treated⁷ postnatal cerebellar cells. Preparation of crude membrane fraction, immunoaffinity purification of antigens on monoclonal antibody columns and Western blot techniques have been described^{4,7}. Immunopurified antigens (visualized by reduced silver staining⁴): lane 1, L1; 2, N-CAM; 3, 'rest L2'. Antigens for Western blots (lanes 4-11): 4 and 7, L1; 5, 8 and 10, N-CAM; 6, 9 and 11, 'rest L2' obtained by sequential immunoaffinity removal of L1 and N-CAM before immunoaffinity chromatography on an L2 antibody column. Antibodies: 4-6, L2 antibody; 7-9, HNK-1 antibody; 10, polyclonal anti-BSP-2/N-CAM antiserum; 11, polyclonal anti-MAG antiserum.

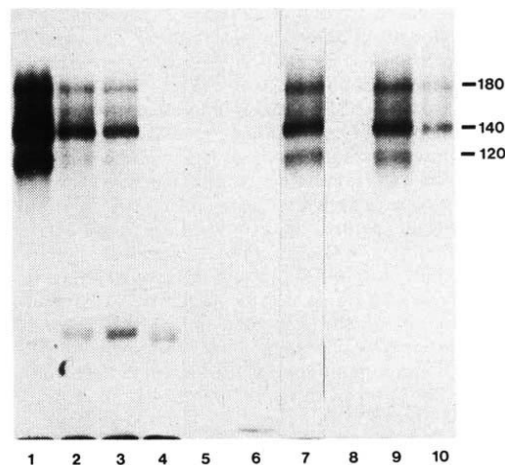


Fig. 2 Sequential immunoprecipitation of immunopurified, iodinated mouse N-CAM with two monoclonal antibodies to N-CAM (H28.123 and P61 (ref. 33) 1, 2, respectively), L2 antibody (3) and L1 antibody (4). Antibody P61 does not recognize the 120 kd band³³; 5, precipitation with H28.123 after preclearing with H28.123; 6, anti-L2 after preclearing with H28.123; 7, H28.123 after preclearing with anti-L2; 8, anti-L2 after preclearing with anti-L2; 9, H28.123 after preclearing with anti-L1; 10, anti-L2 after preclearing with anti-L1. N-CAM was immunopurified from adult mouse brain membrane in presence of 2-deoxy-2,3-dehydro-N-acetylneuraminic acid (Boehringer, Mannheim) to prevent possible degradation of terminal sugars. Since endogenous endoglycosidase activities in eucaryotic cells are negligible, it is unlikely that the N-CAM preparation contains degraded carbohydrate chains. Immunoprecipitation was carried out with mouse anti-rat kappa mouse Ig coupled to Sepharose as described⁷. N-CAM (5×10^5 c.p.m.) was incubated for 1 h at 0 °C with first antibodies and for 4 h at 4 °C with Sepharose beads. After four washings, supernatants from the precipitates were again treated identically with antibodies and Sepharose beads. Treatment of supernatants with Sepharose beads was repeated to remove potentially present soluble complexes.

Table 1 Inhibitory capacity of carbohydrate moiety of 'L2 antigen' for binding of L2 and HNK-1 antibodies to immunopurified 'L2 antigen'

Antibodies	Competing compounds			
	'L2 antigen'		Carbohydrate fraction of 'L2 antigen'	
	Half-maximal inhibition	Maximal inhibition	Half-maximal inhibition	Maximal inhibition
L2	0.176 μ g	1.76 μ g	0.28 μ g	1.4 μ g
HNK-1	0.176 μ g	1.76 μ g	16.8 μ g	ND

An ELISA test²⁹ was performed with L2 and HNK-1 antibodies on immunoaffinity purified 'L2 antigen' (20 ng per spot on nitrocellulose filters) in the presence of competing 'L2 antigen' and carbohydrate fraction obtained from 'L2 antigen' by proteolytic digest. Treatment of 'L2 antigen' (2 mg ml⁻¹) in 50 mM Tris, pH 8.2 containing 0.1% SDS and 4 mM CaCl₂ with pronase (three consecutive additions of 30 μ g ml⁻¹, 30 h, 37 °C), subsequent redigestion with pronase (27 h, 37 °C, 2 mM CaCl₂) or proteinase K (27 h, 37 °C, 10 mM EDTA), isolation by gel filtration on Biogel P4 and chromatographic analysis of carbohydrate fraction were carried out as described elsewhere^{14,30}. Antibodies were used at a concentration of 2 serial dilutions before titre endpoint, 1:200,000 from a 14 mg ml⁻¹ stock for L2 and 1:100,000 for HNK-1 and incubated with 'L2 antigen' or carbohydrate fraction (1 h, room temperature) before the ELISA test. Amounts of competing compounds are given in μ g protein for 'L2 antigen'³¹ and μ g carbohydrate for carbohydrate fraction³² which give half maximal and maximal inhibition of antibody binding. ND, not determined.

type-specific neural markers. In monolayer cultures of early postnatal cerebellar cells the L2 and HNK-1 antibodies reacted with a fraction of tetanus toxin¹⁶, N-CAM and L1 positive neurones, glial fibrillary acidic protein (GFA) and vimentin-positive astrocytes¹⁷ and 04 and 01 antigen-positive oligodendrocytes¹⁸⁻²⁰, but not with fibronectin-positive fibroblast-like cells²¹. The two antibodies were able to block each other in binding. With time in culture the percentage of N-CAM positive neurones that were also L2/HNK-1 positive decreased from 49 $\pm$ 8% or 52 $\pm$ 2% after 1 day to 18 $\pm$ 5% or 13 $\pm$ 3% after 7 days in culture for L2 or HNK-1, respectively.

To investigate the paradoxical finding that only some neurones that are recognized by polyclonal and monoclonal N-CAM antibodies express the L2 epitope, sequential immunoprecipitation of iodinated N-CAM immunopurified from adult mouse brain (see Fig. 1) was performed with antibodies to N-CAM and L2 (Fig. 2). Antibodies to N-CAM removed all further reactivity for the same or for L2 antibody. In contrast, two clearings with anti-L2 removed all material reacting with the same antibody, but left considerable amounts of the 180, 140 and 120 kd bands in solution which could be precipitated by

N-CAM antibodies. L2 antibodies removed only 15-20% of the N-CAM that is precipitable by N-CAM antibodies. Similar results were obtained, when the embryonic form of N-CAM was used for immunoprecipitation (not shown). These results support the conclusion that not all N-CAM molecules of both the adult and embryonic forms express the L2 epitope. Furthermore, the L2 antibody did not bind the 120 kd band (Fig. 2).

Since two bands of N-CAM are heterogeneous with respect to expression of the L2 epitope, the most likely explanation for the absence of L2 on N-CAM bearing neurones is a heterogeneity among N-CAM molecules which is distinct from the molecular heterogeneity represented by the adult and embryonic forms. On the other hand, some neurones may express only the 120 kd component. A similar heterogeneity may also exist for L1, since in this case also not all L1 positive neurones were found to express the L2 epitope.

The present study has shown that two cell adhesion molecules share a common carbohydrate moiety also present on myelin-associated glycoprotein. From our experiments we cannot determine whether the two antibodies recognize the same epitope or two distinct epitopes closely associated with each other on the carbohydrate chains so that the antibodies compete with each other for binding. In any case, the L2/HNK-1 epitopes are simultaneously expressed on the same select group of molecules. It has been suggested that myelin associated glycoprotein mediates the interaction of the periaxonal membrane of myelin with the axon²². It is possible, therefore, that the common moiety is not only a sign of fortuitous structural homologies between neural cell surface constituents, but implicates that the L2 expressing molecules perform similar functional tasks.

The role of the L2/HNK-1 carbohydrate moiety in cell interactions remains at present unknown. Variations in carbohydrate composition of the embryonic and adult forms of N-CAM underlie differences in adhesive properties^{23,24} and it is conceivable that the L2 epitope represents an additional possibility to modulate cell interactions among neural cells. Heterogeneity in expression of the L2 epitope in the population of N-CAM molecules on individual neurones would provide particular cells with the capability to superimpose other tuning modes of adhesion between cell surface receptors and their ligands as they have been described in non-neural systems (see, for instance, ref. 25). Whether adhesive properties are affected in human gammopathies with demyelinating neuropathy and antibodies to myelin associated glycoprotein²⁶⁻²⁸ remains an open question.

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Neuronal-type Na⁺ and K⁺ channels in rabbit cultured Schwann cells

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Nerve axons in the central and peripheral nervous system are normally surrounded by satellite cells. These cells, known as Schwann cells in the peripheral nervous system, interact with axons to form a myelin sheath, so allowing nerve impulses to proceed at high speed. Schwann cells are thought to differ from neurones in their membrane properties in one important aspect: they lack excitability. Using the patch-clamp technique we have now measured directly the ionic currents across the membrane of single Schwann cells cultured from newborn rabbits. Surprisingly, we found that these Schwann cells possess voltage-gated sodium and potassium channels that are similar to those present in neuronal membranes.

Figure 1 shows 2-day-old Schwann cells cultured from the sciatic nerves of newborn rabbits¹. Although markers (such as RAN-1) exist for rat Schwann cells, no such marker is readily available for the rabbit. Schwann cells in the present study were therefore identified morphologically by their characteristic bipolar spindle shape; this is distinct from the flat multipolar appearance of the contaminating fibroblasts that form the only other cellular component of the primary cultures used (and which were not studied electrophysiologically in the present experiments). Although Schwann cells in culture may come to resemble fibroblasts in shape in certain conditions², fibroblasts are unlikely to be mistaken for Schwann cells. Proliferation of fibroblasts was inhibited in some cultures by adding cytosine arabinoside to the medium.

Ionic currents were recorded using the patch-clamp technique of Hamill *et al.*³ in the whole cell configuration. Following formation of a gigohm seal to the cell surface, the patch membrane was broken by excess suction, allowing access to the cell interior. Patch pipettes were filled with solutions in which the primary salt was KCl, CsCl or CsF, together with Ca²⁺ buffered to low concentrations by EGTA. In this system, ion concentrations inside the cell equilibrate with those in the pipette with a time constant of about 5 s (ref. 4). Over 60 cells were studied in cultures for 2–10 days.

Figure 2a shows ionic currents recorded from a rabbit Schwann cell with KCl in the patch pipette. The cell was held at -75 mV and test pulses, each preceded by a prepulse to -115 mV, were applied in 10-mV increments, the final potential ranging from -75 mV to +25 mV. The records consist of two components, an early inward current and a delayed outward current. They thus resemble responses found in a number of

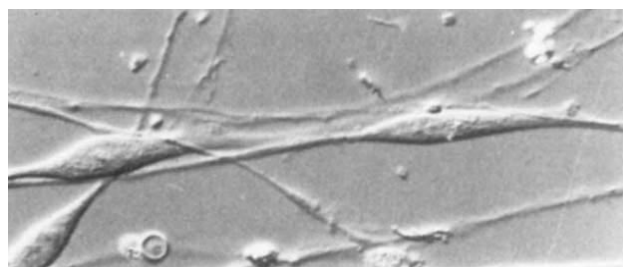


Fig. 1 Two-day-old cultured rabbit Schwann cells under Nomarski optics. (×610).

neurones. We have used pharmacological compounds of known specificities to characterize these Schwann cell currents further. Figure 2b shows the effect of the K⁺ channel blocker 4-aminopyridine (4-AP)^{5,6}. Two minutes after adding 6 mM 4-AP to the solution bathing the cell, the delayed outward current was selectively blocked. In other experiments in which the pipette was filled with CsCl, another blocker of K⁺ channels, only inward currents were observed. We thus conclude that the delayed outward current is a K⁺ current similar to that found in squid and frog nerves.

The nature of the initial inward current was studied with tetrodotoxin (TTX), a highly selective blocker of Na⁺ channels⁷. Figure 3 shows inward currents before (a) and 30 s after (b) the introduction of 60 nM TTX into the bathing solution. Potassium currents in this cell had been eliminated by using a pipette containing CsF. It can be seen that the transient inward current was virtually abolished by TTX. Figure 3c illustrates a family of ionic currents which includes depolarizations sufficiently strong to elicit outward transient currents. The pipette solution contained primarily CsCl to block current through K⁺ channels but included 29 mM Na⁺. We consistently found that the apparent reversal potential was about +70 mV, which is about 30 mV more positive than the Na⁺ equilibrium potential. There are at least two possible explanations for this discrepancy. First, most of the steady-state current in Fig. 3c was not blocked by TTX or Cs and may thus be flowing through a third ionic channel. This suggestion is supported by the recent findings of anionic¹ and non-selective cationic⁸ channels in rat Schwann cells; and we have found single channels in rabbit cells which appear similar to these but will require further characterization. This current would have little effect on our estimate of maximum peak inward current, but might be significant at +70 mV. A second possibility is that the spindle shape of these cells may lead to spatial non-uniformity and poor control of the internal potential by the voltage-clamp.

Although all cells exhibited Na⁺ currents, the amplitude of the currents varied considerably among different cells (compare Figs 2a and 3a). For example, the maximum peak inward Na⁺ current ranged from 50 to 2,100 pA, with an average value of 498 ± 84 pA (±s.e.m., n = 28). This variation did not seem to be

Fig. 2 Ionic currents in 8-day-old cultured rabbit Schwann cells in normal Locke's solution. Records were made using the whole cell configuration of the patch-clamp technique before (a) and after (b) adding 6 mM 4-aminopyridine externally. The patch pipette was filled with (mM): KCl, 140; CaCl₂, 1; MgCl₂, 2; EGTA, 11; HEPES, 10; NaOH, 29 to pH 7.2. The Locke's solution contained (mM): NaCl, 154; KCl, 5.6; CaCl₂, 2.4; MOPS, 10; pH 7.4. Test depolarizations were applied in 10-mV steps from -75 mV to +25 mV. The cell was held at -75 mV and 300-ms prepulses to -115 mV preceded each test step. Linear contributions to leak and capacitive currents were removed by scaling and adding the average of several traces elicited by a hyperpolarization to -120 mV. Currents were sampled and digitized at 50-μs intervals. Temperature, 23 °C.

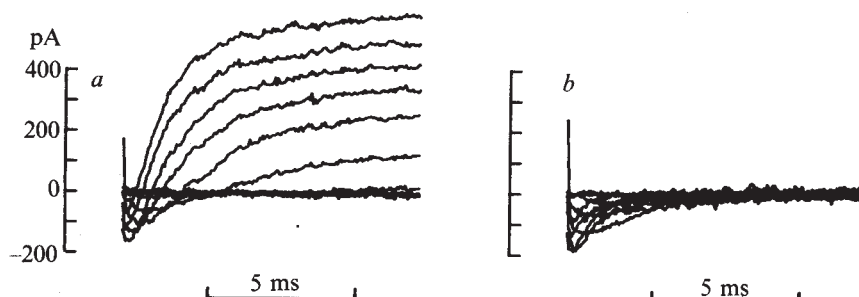
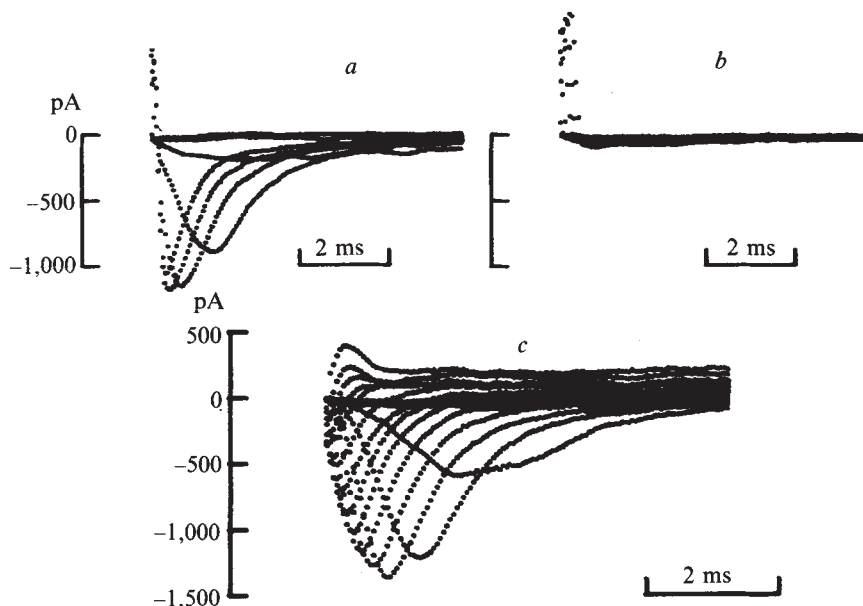


Fig. 3 Ionic currents in 7-day-old cultured rabbit Schwann cells in normal Locke's solution obtained before (a) and after (b) addition of 60 nM TTX externally. The patch pipette was filled with (mM): CsF, 140; CaCl₂, 1; MgCl₂, 2; EGTA, 11; HEPES, 10; NaOH, 29 to pH 7.2. Holding potential, -74 mV. Prepulses to -114 mV were applied for 300 ms. Test depolarizations were made in 10-mV steps to final potentials ranging from -64 mV to -4 mV. c, A family of ionic currents recorded from another (3-day-old) Schwann cell. The feedback resistor in the patch-clamp headstage was reduced to 1 G Ω to allow strong test pulse depolarizations. The pipette solution was identical to that of a, b except that CsCl was used in place of CsF. The cell was held at -75 mV. Test pulses were applied in 10-mV steps from -55 to +105 mV and were preceded by 300-ms hyperpolarizations to -115 mV. The external medium was normal rabbit Locke's solution.



correlated with the age of the culture. Other factors, such as cell shape or phase of the mitotic cycle, were not examined. The cell capacitance was measured in several experiments by integrating the change in the capacity transient on rupture of the patch membrane. The average value was 20 ± 2 pF ($n = 14$) and the corresponding peak current 30 ± 9 pA pF⁻¹ ($n = 14$). Examination of a typical Schwann cell under light microscopy showed the cell to be spindle shaped, about 50 μ m from pole to pole, and about 10 μ m in diameter in the middle. If any contribution from the small processes at either end are ignored, an upper limit of about 3 μ F cm⁻² is obtained for the membrane capacity of the Schwann cell.

Ritchie and Rang⁹ have recently measured the uptake of tritiated saxitoxin (STX) by rat and rabbit sciatic nerves that had undergone axonal degeneration resulting from excision of a small segment of the trunk. In peripheral nerves of rabbit, but not rat, the saturable binding of STX (which blocks Na⁺ channels in a manner similar to that of TTX) increases dramatically from about 5 days after sectioning, a time coincident with the known proliferation of Schwann cells in regions distal to the excision. The results presented here thus provide strong support for the idea that these STX binding sites are indeed voltage-sensitive Na⁺ channels of the kind generally believed to be restricted to excitable tissue. Glial cells (and fibroblasts) are already known to possess channels that are opened by veratridine and blocked by TTX^{10,11}. However, in such experiments there is no evidence of voltage sensitivity; and, in fact, glial cells in particular have long been thought to be inexcitable. Intracellular recording from satellite cells in the leech central nervous system¹² and the squid giant axon¹³ has failed to detect any regenerative response. Furthermore, *Necturus* central glia lack a specific, saturable component of binding of STX¹⁴. By contrast, rabbit Schwann cells do seem to possess the machinery necessary for action potential generation. However, their resting potential, which we have measured to be about -30 to -40 mV, probably inactivates most Na⁺ channels, rendering these cells effectively inexcitable. Whether these Na⁺ and K⁺ channels are included in the myelin lamellae generated by Schwann cells, whether they have any role in remyelination or axonal regeneration, or whether they are conceivably a source of axolemmal Na⁺ (and K⁺) channels, pose interesting questions for future investigation.

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Photoreceptor excitation and adaptation by inositol 1,4,5-trisphosphate

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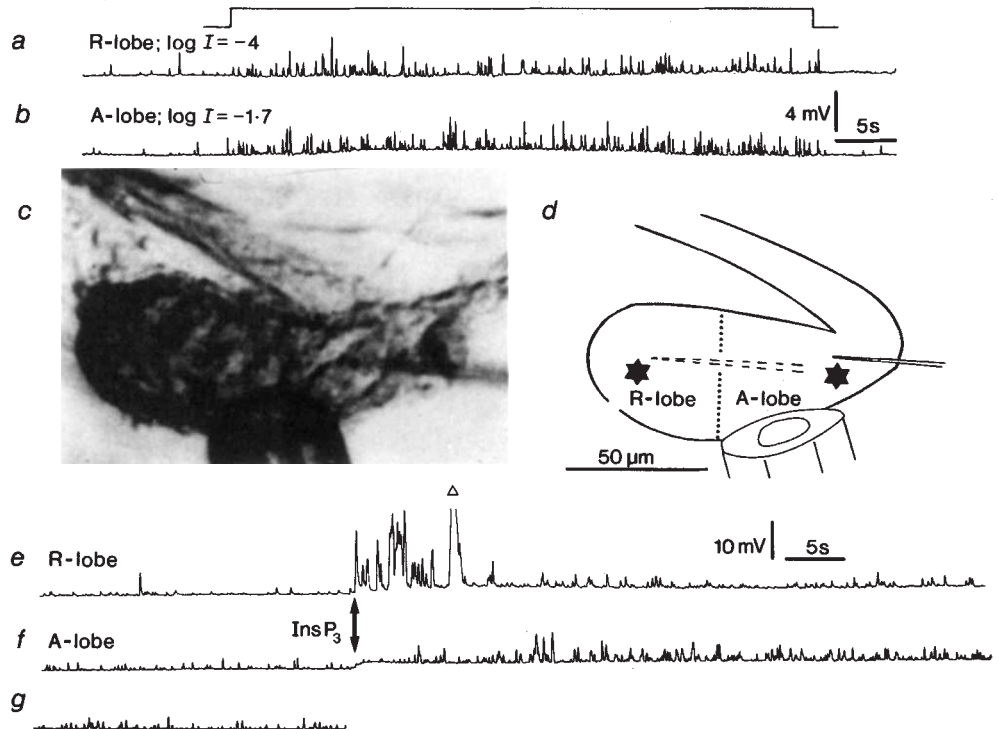
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A central question concerning vision is the identity of the biochemical pathway that underlies phototransduction. The large size of the ventral photoreceptors of *Limulus polyphemus* renders them a favourite preparation for investigating this problem¹. The fact that a single photon opens approximately 1,000 ionic channels in these photoreceptors^{2,3} suggests the need for an internal transmitter. We have investigated whether inositol 1,4,5-trisphosphate (InsP₃) functions as such an internal transmitter, given that InsP₃ may act as an intracellular messenger in other cellular processes^{4,5}. Here we report that in *Limulus*, intracellular pressure injection of InsP₃ both excites and adapts ventral photoreceptors in a manner similar to light.

Fig. 1 InsP_3 -induced excitation of *Limulus* ventral photoreceptor. The photomicrograph (c) shows the receptor after the connective tissue had been stripped away¹¹. The tip of the suction pipette, which was used for stripping and holding the cell, can be seen in the micrograph. Recordings and injections were made through a pipette, filled with a stock solution containing (in mM): 100 K aspartate, 10 HEPES, pH 7.0 in which 20 μM InsP_3 was dissolved. Injections of stock solution alone were found to have no effect (30 cells). The cell was first impaled in the A-lobe and the extent of the A-lobe and R-lobe determined by scanning the cell with a 10- μm spot of light⁹. Records a and b show the response of the R-lobe and A-lobe respectively to steady illumination (square pulse at the top of the traces) with a spot of the indicated $\log_{10}$ attenuation from the maximum available intensity. The R-lobe was found to be approximately 200 times more sensitive to light than the A-lobe¹¹. Record f shows the response to an InsP_3 injection (15 p.s.i., 200-ms pulse at the arrow) into the A-lobe and record g shows the recovery 150 s after the injection. Following this injection, the same electrode was inserted into the R-lobe. Sensitivity in the R-lobe was then checked and found to be unchanged relative to a. Record e shows the response to an InsP_3 injection (15 p.s.i., 200-ms pulse at the arrow) into the R-lobe. (The open triangles in the records of this figure and the solid ones in subsequent figures indicate when the recordings went off the scale of the chart recorder.) The sketch of the cell in d shows the location of the pipette during injections into the R-lobe and A-lobe. $\star$ Indicates the location of the spot of light, when centred on the A- and R-lobes. The dotted line indicates the probable demarcation of the R-lobe and A-lobe, as determined by a large change in sensitivity to a spot of light. After record e was obtained, the electrode was withdrawn and the A-lobe re-impaled. Results similar to f were again obtained (not shown). Similar results were obtained in nine other cells. InsP_3 , $\text{Ins}(1,4)\text{P}_2$ and $\text{Ins}(4,5)\text{P}_2$ were prepared essentially by the method of Grado and Ballou²¹ as adapted by R.F.I., M.J.B. and K. D. Brown (in preparation). Briefly, a crude inositol fraction from ox brain was hydrolysed by 1M NaOH and, after neutralization with formic acid, the hydrolysate was fractionated into crude InsP_1 , InsP_2 and InsP_3 on Dowex-formate columns²². InsP_3 , $\text{Ins}(1,4)\text{P}_2$ and $\text{Ins}(4,5)\text{P}_2$ were further purified by paper chromatography²¹ and their identities and purities checked by ionophoresis. A blank region from a paper chromatograph was also eluted and, like the inositol phosphates, de-cationized, followed by neutralization with KOH.



We have previously described the methods for intracellular recording, stimulating and reliably pressure-injecting aliquots of solution of the order of 10 pl (refs 6–9). Injected solutions are specified by the concentration in the pipette. InsP_3 , inositol 1,4-bisphosphate ($\text{Ins}(1,4)\text{P}_2$) and inositol 4,5-bisphosphate ($\text{Ins}(4,5)\text{P}_2$) were prepared as described in Fig. 1 legend; *myo*-inositol, potassium phosphate, *myo*-inositol 2-monophosphate and phytic acid (inositol hexaphosphoric acid) were from Sigma.

The ventral photoreceptors of *Limulus* are segmented into two lobes, a rhabdomeral lobe (R-lobe), specialized for photo-transduction, and an arhabdomeral lobe (A-lobe)^{10,11}, the R-lobe being approximately 100 times more sensitive to light than the A-lobe (Fig. 1a, b)¹¹. When injected into the R-lobe, 20 μM InsP_3 reversibly induced, with no detectable latency, the production of discrete waves of depolarization and bursts of waves (Fig. 1e). The individual InsP_3 -induced waves have a similar waveform to the 'quantum bumps' that are evoked by single photons (Fig. 1a). Injection of InsP_3 into the A-lobe resulted in a response that was both greatly delayed and much smaller (Fig. 1f, g), as would be expected if the injected InsP_3 had to diffuse into the R-lobe in order to produce an effect. The greater sensitivity to InsP_3 of the R-lobe, coupled with a similar effect to that of light, suggests that InsP_3 acts on the structures of the R-lobe that are specialized for phototransduction.

When measured under voltage clamp, the response to InsP_3 (Fig. 2a, c) is an inward current, as is the light response (Fig. 2b). When cells were stimulated with a dim flash (Fig. 2d, arrow) followed by an injection of InsP_3 (Fig. 2d, solid bar), the currents due to light and InsP_3 both reversed at approximately +18 mV; this result would be expected if light and InsP_3 opened the same ionic channels. Light-adaptation by a bright flash desensitizes

the photoreceptor and decreases the InsP_3 -induced current (Fig. 2a–c); thus, the response to InsP_3 can be light-adapted.

The specificity of the excitatory effect of InsP_3 was checked by injecting related compounds. The following had no effect: *myo*-inositol (1 mM, 11 cells), phosphate (1 mM, 10 cells), *myo*-inositol-2-monophosphate (1 mM, 9 cells), phytic acid (1 mM, 6 cells) and a blank from the paper chromatograph (10 cells) (see Fig. 1 legend). $\text{Ins}(1,4)\text{P}_2$, the degradation product of InsP_3 , had no effect at concentrations of 20 μM (14 cells) and 100 μM (12 cells); however, two out of three cells exhibited an excitatory effect when 500 μM $\text{Ins}(1,4)\text{P}_2$ was injected. $\text{Ins}(4,5)\text{P}_2$ had an excitatory effect at 20 μM (9 cells), indicating that the site of InsP_3 action is not selective for InsP_3 over $\text{Ins}(4,5)\text{P}_2$.

As InsP_3 excites the receptor, it might initiate other processes that follow excitation, such as adaptation. Figure 3a shows localized adaptation by light, in which a prolonged stimulus at position 1 reduced subsequent responses at this position more than at position 2. Similarly, injecting InsP_3 at position 1 reduced subsequent responses to light flashes at this position more than at position 2 (Fig. 3c, d). Desensitization following InsP_3 injection under voltage clamp is shown in Fig. 3e. We have also observed desensitization of responses to InsP_3 following injection of InsP_3 (not shown). We have not seen in any cell an InsP_3 -induced desensitization that was not preceded by excitation resulting from InsP_3 injections. The similarity of the desensitizing effect of InsP_3 to that of light suggests that InsP_3 , or a related compound, initiates processes leading to adaptation as well as to excitation.

A rise in intracellular calcium has been implicated in the adaptation of ventral photoreceptors¹². The desensitizing effect of InsP_3 injection shown in Fig. 3c–e might be caused by calcium

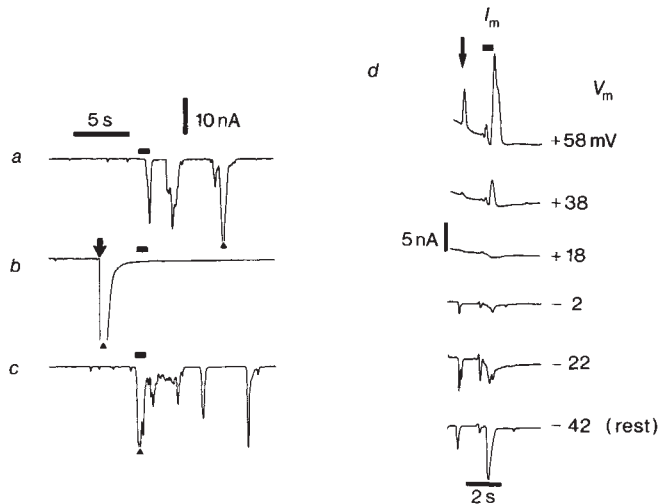


Fig. 2 Light adaptation and reversal potential of InsP_3 -induced currents. The currents in *a*, *b*, and *c* were obtained from a cell voltage-clamped to its membrane potential (-50 mV) in the dark. The bar above the records indicates the duration of injection with $20 \mu\text{M}$ InsP_3 . The arrow above the records indicates a 20-ms flash of uniform white light attenuated by 1.5 log units in *b* and by 5.0 log units in *d*. In *b*, light adaptation blocks the InsP_3 response. Spontaneous quantum bumps are present in record *b* before the flash, but not after, indicating that the cell was adapted by the flash. The record in *c* was obtained after the cell recovered sensitivity, following the adapting flash in *b*. A video recording of the injection made under IR illumination⁸ allows us to determine that a successful injection occurred in *b* even though there was no effect of the injection. Record *d*, taken from a different cell, shows that the reversal potential of the InsP_3 -induced current coincides with that of the light-induced current. These voltage-clamp recordings were made in artificial seawater in which the Ca^{2+} was reduced from 10 mM to 1 mM to avoid desensitization produced by voltage-dependent Ca^{2+} entry²³. The membrane potential (V_m) was moved in 20-mV steps through the reversal potential by a series of 15 s-long command pulses, each beginning 10 s before the test flash. The large steady-state component of the transmembrane current resulting from the voltage step was subtracted from the current records.

contamination of the injection solution; de-cationization of the InsP_3 preparation (see Fig. 1 legend) minimized such contamination. Also, six cells were injected with $100 \mu\text{M}$ InsP_3 to which $500 \mu\text{M}$ EGTA had been added to reduce the free Ca^{2+} to $<10^{-7}$ M; this solution produced the same effects as in Fig. 3 *c–e*. Therefore, it is unlikely that the observed desensitization is due to calcium contamination of the injection solution.

Illumination can facilitate or enhance a photoreceptor's response to a flash of light^{7,13–15}. Figure 3*d* shows that the light response at spot 2 was transiently facilitated immediately after InsP_3 injection. At a site (spot 1) closer to the injection, this facilitation may be masked by adaptation (Fig. 3*c*). The voltage-clamp recording in Fig. 3*f* was obtained from another cell to demonstrate that the InsP_3 -induced facilitation could be experimentally separated from InsP_3 -induced desensitization. Multiple injections of InsP_3 produced an approximately threefold facilitation of the light response. Comparison of Fig. 3*e* and *f* illustrates our notion that a sustained facilitation of the light response is associated with a weaker excitation than accompanies desensitization. InsP_3 or a related compound may, therefore, initiate facilitation as well as excitation and adaptation.

It is unlikely that InsP_3 is the sole messenger in the pathway of adaptation and excitation. Light is thought to release calcium from internal stores so as to adapt the light response in ventral photoreceptors^{12,16}. It has been suggested that InsP_3 causes the release of calcium from an intracellular compartment in pancreatic acinar cells⁵ and in hepatocytes^{17,18}. InsP_3 might, therefore, release calcium from intracellular stores in ventral photoreceptors to induce adaptation. The inability of $500 \mu\text{M}$ EGTA, injected with $100 \mu\text{M}$ InsP_3 , to block desensitization may be due to the inability of this amount of EGTA to significantly

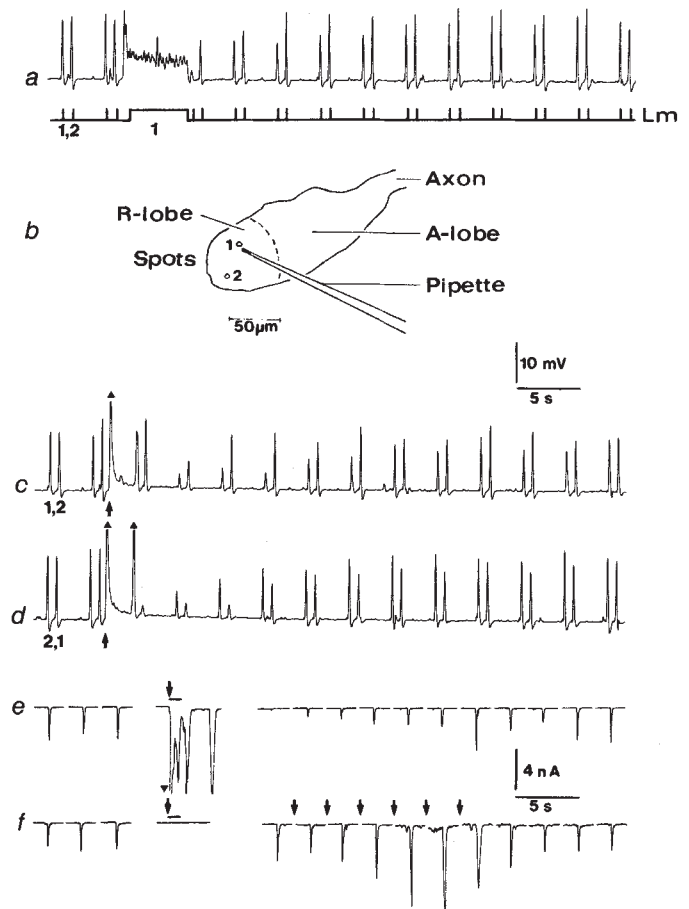


Fig. 3 InsP_3 -induced desensitization. Localized desensitization was produced both by light (*a*) and by injection of $100 \mu\text{M}$ InsP_3 (*c* and *d*) in a cell whose outline is shown in *b*. In record *a*, the light monitor (Lm) denotes the time of occurrence of the light stimuli at spots 1 and 2. Record *a* demonstrates that light adaptation at spot 1 produces a stronger desensitization of subsequent responses at spot 1 than at spot 2 (ref. 9). Similarly, injection of $100 \mu\text{M}$ InsP_3 at spot 1 produces a greater desensitization of subsequent responses at spot 1 compared with spot 2 (records *c* and *d*). The sequence of light flashes in *c* and *d* was reversed to exclude the possibility that the localized desensitization was in any way related to the sequence in which the two regions of the cell were illuminated. Similar findings were obtained in two other cells with injections of $100 \mu\text{M}$ InsP_3 and in three further cells with $100 \mu\text{M}$ InsP_3 and $500 \mu\text{M}$ EGTA (see text). The same experiment was performed with $20 \mu\text{M}$ InsP_3 ; three out of four cells showed a localized desensitization. As controls, four cells were injected with $20 \mu\text{M}$ $\text{Ins}(1,4)\text{P}_2$; none showed a localized desensitization. Of six cells injected with $100 \mu\text{M}$ $\text{Ins}(1,4)\text{P}_2$, none showed localized desensitization for single injections. Multiple injections, however, did induce a localized desensitization in two of these cells. The voltage-clamp recording in *e*, from another cell, demonstrates InsP_3 -induced desensitization of the light response following the excitatory effect of InsP_3 injection (arrow). Record *f* demonstrates facilitation of the light response following multiple injections (arrow) of InsP_3 into another voltage-clamped cell; in this cell, the electrode was probably in the A-lobe as there was only a slowly developing excitatory effect with each InsP_3 injection (not shown). In *e* and *f*, the bars indicate the injection duration; the test flash was 20 ms, the intensity was attenuated by 5.0 log units and the illumination was uniform. A portion of the record between each light response was deleted in *e* and *f* for ease of presentation.

increase the existing calcium buffering capacity of the cell. Further investigation is needed to determine whether InsP_3 directly releases calcium from within the cell.

Although InsP_3 may adapt the receptor by means of stimulating the release of calcium, it is unlikely that the excitatory effect of InsP_3 is mediated solely by a rise in calcium. Ionophoretic injection of calcium does not excite ventral photo-

receptors¹². In addition, we must also explain the correlated opening of the thousands of channels that underlie the InsP_3 -induced discrete waves and bursts. If these channels open independently of each other then InsP_3 must initiate the correlated release of many molecules of another transmitter which opens the individual channels.

If visual excitation and adaptation involve InsP_3 , one might reasonably expect that the level of InsP_3 , and of its precursor, phosphatidylinositol 4,5-bisphosphate [$\text{PtdIns}(4,5)\text{P}_2$], would

change during illumination. Light causes a drop in the incorporation of ^{32}P into $\text{PtdIns}(4,5)\text{P}_2$ of the octopus eye¹⁹ and the 'no receptor potential' (norp A) mutant of *Drosophila* exhibits increased incorporation of ^{32}P into $\text{PtdIns}(4,5)\text{P}_2$ (ref. 20). Our physiological findings taken together with these biochemical data, suggest an important role for InsP_3 in phototransduction.

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myo-inositol polyphosphate may be a messenger for visual excitation in *Limulus* photoreceptors

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Photoreceptor excitation begins with the absorption of a photon by rhodopsin and proceeds through an unknown sequence of steps that leads to changes in specific ionic conductances. These conductance changes produce the receptor potential. It has been proposed^{1–3} that hydrolysis of phosphoinositides is involved in the control of a variety of physiological processes. Recent studies have implicated inositol 1,4,5-trisphosphate as an intracellular messenger in the cascade mediating hormone-stimulated secretion^{4–7}. We propose that one of the steps in the excitatory cascade^{8,9} in *Limulus* ventral photoreceptors may be an increase in intracellular concentration of myo-inositol polyphosphates, derived from hydrolysis of the membrane component phosphatidylinositol bisphosphate by a phospholipase. Here we present biochemical and electrophysiological evidence that an inositol polyphosphate may be an intracellular messenger in the cascade mediating excitation, based on the following criteria: (1) the cells possess the synthetic and degradative metabolism for the messenger; (2) the natural stimulus leads to a change in the concentration of the messenger within the cells; and (3) intracellular injection of exogenous messenger mimics naturally occurring electrophysiological events.

Limulus ventral rudimentary eyes^{10,11} were dissected out of the blood vessels that ensheath them. The cluster of photoreceptor cell bodies at the distal end of the nerve was assayed for the effects of illumination on phosphoinositide metabolism. Steady illumination increased the incorporation of $^{32}\text{PO}_4$ and ^3H -inositol into phosphatidylinositol (compare the light/dark ratio for paired ventral eyes, Table 1). However, the amount of ^3H -inositol incorporated into phosphatidyl inositol bisphosphate (PtdInsP_2) decreased with steady illumination while there was no effect on $^{32}\text{PO}_4$ incorporation (Table 1). Fisher and Agranoff¹² reported a similar finding from synaptosomes in

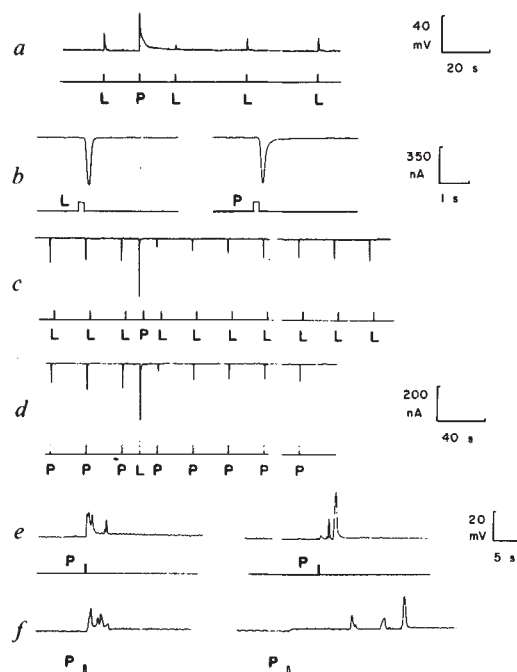


Fig. 1 Electrophysiological effects of intracellular injections of inositol polyphosphates. *Limulus* ventral photoreceptors were impaled with either one micropipette to measure membrane voltage³⁵ or two micropipettes to measure voltage-clamp currents^{36,37}. The electrodes were inserted into the rhabdomeric lobe except where noted. The lower of each pair of traces is a monitor for stimuli: L denotes illumination. Injections (denoted P) were made by applying pressure to the back of the micropipette. *a*, Membrane voltage recorded from an electrode filled with 400 μM $\text{Ins}(1,4,5)\text{P}_3$ plus 200 mM KCl. Light intensity $3 \times 10^{-7} \text{ W cm}^{-2}$. Pressure injections of $\text{Ins}(1,4,5)\text{P}_3$ induced large depolarizations and attenuated the subsequent responses to illumination. *b–d*, Currents recorded by a voltage clamp. *b*, Light stimulus $2.1 \times 10^{-5} \text{ W cm}^{-2}$, duration 200 ms. *c*, Note that pressure injection of $\text{Ins}(1,4,5)\text{P}_3$ induced an inward current and desensitized the light response (light intensity $1.3 \times 10^{-6} \text{ W cm}^{-2}$). *d*, Conversely, light ($1.3 \times 10^{-3} \text{ W cm}^{-2}$) desensitized the response of the photoreceptor to the injection of $\text{Ins}(1,4,5)\text{P}_3$ (same scales for *c* and *d*). Records *a–d* were from the same cell. *e, f*, Membrane voltage recorded by micropipettes filled with 400 μM $\text{Ins}(1,4,5)\text{P}_2$ in each of two cells. The left-hand records were obtained with the micropipettes placed in the rhabdomeric lobe; the right-hand records were obtained with the micropipette placed near the rhabdomeric lobe (in *e*) and far from the rhabdomeric lobe (in *f*). Note that the $\text{Ins}(1,4,5)\text{P}_2$ -induced depolarizations appear to be summated 'discrete events'^{21,22} and are more delayed when the injection pipette is impaled farther from the rhabdomeric lobe (same scales for *e* and *f*).

Table 1 Effect of light on the incorporation of labelled precursors into phosphoinositides

	Precursor	Product	Total d.p.m. radioactivity (mean $\pm$ s.e.m.)		Ratio light/dark per animal (mean $\pm$ s.e.m.)	P value for ratio
			Light	Dark		
a	^3H -inositol	InsP ₃	930 $\pm$ 157	563 $\pm$ 122	1.76 $\pm$ 0.22	0.005 (25)
b	^3H -inositol	PtdIns	3,660 $\pm$ 388	3,232 $\pm$ 345	1.20 $\pm$ 0.09	0.05 (10)
	^3H -inositol	PtdInsP	344 $\pm$ 56	456 $\pm$ 50	0.84 $\pm$ 0.16	>0.1 (10)
	^3H -inositol	PtdInsP ₂	1,928 $\pm$ 262*	3,001 $\pm$ 289*	0.66 $\pm$ 0.09	0.01 (10)
	$^{32}\text{PO}_4$	PtdIns	4,558 $\pm$ 583*	2,008 $\pm$ 277*	2.37 $\pm$ 0.23	0.01 (10)
	$^{32}\text{PO}_4$	PtdInsP	710 $\pm$ 183	546 $\pm$ 57	1.46 $\pm$ 0.45	>0.4 (10)
	$^{32}\text{PO}_4$	PtdInsP ₂	3,466 $\pm$ 545	3,425 $\pm$ 411	1.01 $\pm$ 0.17	>0.5 (10)

a, Isolated ventral eyes were incubated on three separate occasions in the dark at 21 °C for 6–8 h in *Limulus* serum containing [^3H]-inositol (300–500 $\mu\text{Ci ml}^{-1}$). Each ventral eye was washed twice with artificial seawater²³. One ventral eye of each pair was exposed to light ($1.3 \times 10^{-3} \text{ W cm}^{-2}$) for 30 s, followed immediately by the addition of chloroform/methanol (1:2, v/v). The dark-maintained ventral eye from the same animal was extracted in the same way. Water-soluble inositol phosphates were separated by either ion-exchange chromatography²⁸ or TLC on PEI cellulose²⁹ and counted for radioactivity. Statistical comparisons were made between the ventral eyes from the same animal using a paired Student's *t*-test. b, Each isolated ventral eye was incubated in 0.1 ml *Limulus* serum containing [^3H]-inositol (500 $\mu\text{Ci ml}^{-1}$) and $^{32}\text{PO}_4$ (250 $\mu\text{Ci ml}^{-1}$) in light or dark at 21 °C for 3 h. The incubation was stopped by adding 2 ml of chloroform/methanol/HCl (100:100:6, by vol.), followed by 0.3 ml of 0.2 M HCl. The lower phase was evaporated to near dryness. Phospholipids were separated by TLC using silica gel HR plates in a solvent system of chloroform/methanol/methylamine (40%)/water (60:36:5:5, by vol.). Areas that contained ^{32}P were identified by autoradiography on X-ray film, scraped off and counted. When the total radioactivities from each group were averaged, only those dark/light values marked by an asterisk were significantly different (at the 0.02 confidence level, two-tailed *t*-test). These data are confounded by large between-animal variations. To minimize the effects of these variations, comparisons of light–dark differences in the incorporation of label were made between the ventral eyes isolated from the same animal, using a paired Student's *t*-test (the number of pairs is given in parentheses).

which acetylcholine stimulated $^{32}\text{PO}_4$ incorporation into phosphatidylinositol but reduced the incorporation into polyphosphoinositides. In addition, Yoshioka *et al.* (in *Octopus*)¹³ and Vandenberg and Montal (in squid)¹⁴ have shown that light causes a reduction of $^{32}\text{PO}_4$ incorporation into polyphosphoinositides in preparations of broken photoreceptor cells. We interpret our findings as evidence that phosphoinositides are synthesized in the photoreceptor cells and that the turnover of phosphatidylinositol is increased by illumination, but that the pool of PtdInsP₂ is reduced due to enzymatic release of inositol trisphosphate (InsP₃) into the cytosol. We found that dark-adapted *Limulus* ventral eyes contained InsP₃ and that the content of InsP₃ was increased by illumination (Table 1). Taken together, these data show that *Limulus* ventral eyes contain metabolic mechanisms for the synthesis of polyphosphoinositides and for the light-induced increase in the concentration of InsP₃. Therefore, *Limulus* photoreceptors contain the metabolic mechanisms necessary for the synthesis of the putative excitatory messenger, InsP₃.

We examined the electrophysiological effects of intracellular injection of inositol phosphates into single *Limulus* ventral photoreceptors. Photoreceptors whose cell bodies are isolated from neighbouring photoreceptor cell bodies are found scattered along the ventral eye nerve^{15,16}. The adhering glia and axons were peeled away from these cells by the procedure of Stern *et al.*¹⁷. The location of the rhabdomeric lobe of a peeled cell can usually be identified visually; the rhabdomeric lobe is the region of the cell that contains most of the rhodopsin¹⁸ (G. Murray, personal communication) and which shows the maximum sensitivity to light¹⁷. It is the region of the cell in which both excitation and adaptation occur^{17,19}.

The rhabdomeric lobe was impaled with a micropipette containing InsP₃ (0.004–0.4 mM). The location of the micropipette was viewed using an IR television camera. Successful intracellular injection of a solution caused a local disturbance in optical transmission through the cell, which could be observed on a television monitor²⁰. Brief pressure injections of the InsP₃ solution elicited a depolarization (in 48 cells) that resembled the response of the cell to a flash of light (Fig. 1a and Table 2). Moreover, brief injections of InsP₃ elicited an inward positive membrane current measured by a voltage-clamp technique (19 cells) (Fig. 1b). Injections of some samples of InsP₃ elicited responses that appeared to be the summation of discrete events^{21,22} (28 cells) (Table 2); injections of other samples most often elicited smooth responses. The causes of these differences between samples of InsP₃ are unknown. Injection of InsP₃ was

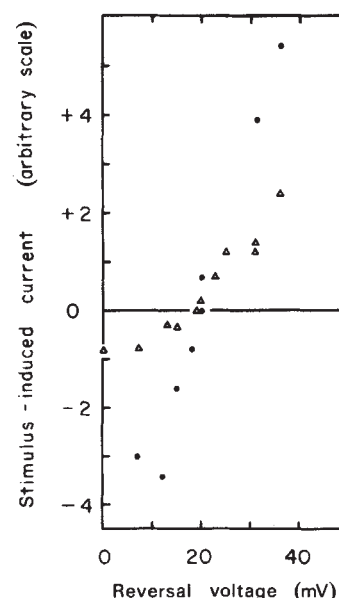


Fig. 2 Reversal voltages of light-induced and Ins(1,4,5)P₃-induced currents. The amplitude of currents recorded by a voltage clamp are plotted against membrane voltage. Note that the two nonlinear curves cross the zero-current axis at approximately the same voltage; that is, the reversal voltage for light-induced currents (●) is approximately the same as that for Ins(1,4,5)P₃-induced currents (Δ).

found to cause a subsequent decrease in the sensitivity of the photoreceptor to light (Fig. 1a, c). Furthermore, light caused a subsequent attenuation in the responses elicited by repetitive injections of InsP₃ (22 cells) (Fig. 1d), that is, light caused an apparent adaptation of the responses elicited by InsP₃ (and vice versa). Injection of InsP₃ into the cell at some distance from the rhabdomeric lobe sometimes elicited little or no smooth depolarization (or smooth inward positive membrane current), sometimes elicited delayed, summated discrete events such as those evoked by the absorption of a small number of photons^{21,22}, but often led to a profound desensitization of the cell.

Injection of inositol 1,4-bisphosphate [Ins(1,4)P₂] or inositol 4,5-bisphosphate [Ins(4,5)P₂] (Table 2) into the rhabdomeric lobe of *Limulus* photoreceptors also induced transient depolarizations. These responses sometimes appeared to be identical to

Table 2 Effects of intracellular injections of *myo*-inositol phosphates

Substance injected	Effect Smooth depolarization or inward current	Discrete events	Total number of cells
400 μ M Ins(1,4,5)P ₃ * (Irvine; human red cell ghosts)	+++ (19)	+	20
40–400 μ M Ins(1,4,5)P ₃ † (Irvine; bovine brain)	+++ (11)	–	11
4 μ M Ins(1,4,5)P ₃ † (Irvine; bovine brain)	–	–	5
2 mM InsP ₃ ‡ (Tarver; bovine brain)	+++ (25)	+++ (27)	27
400 μ M Ins(1,4,5)P ₃ + Ins(2,4,5)P ₃ †§ (Irvine; bovine brain)	+++ (2)	–	2
0.4–4.0 mM Ins(1,4)P ₂ † (Irvine; bovine brain)	++ (3)	++ (6)	8
0.4–4.0 mM Ins(4,5)P ₂ † (Irvine; bovine brain)	++ (9)	++++ (14)	15
Chromatography blank (Irvine; elution from paper)	–	–	5
1.6 mM glycerol-P-Ins(4,5)P ₂ ¶ (Ballou; bovine brain, deacylation)	+++ (9)	+++ (10)	12
400 μ M DL-InsP ₁ **¶ (Sherman; synthetic)	–	–	4
4 mM InsP ₆ †† (Sigma; corn)	–	–	6

The concentrations of compounds shown were those contained in the injection pipettes. The volumes of injections were estimated to be always less than 1% of the cell volume. For each compound, the person who prepared the substance and the source used are given. The numbers in parentheses are the number of cells from which each type of event was observed; some cells showed both types of events. The number of 'plus' signs indicates the relative efficacy of the injections; – indicates no effect.

* Potassium salt prepared as described by Streb *et al.*⁵

† Potassium salt prepared by the method of Grado and Ballou³⁰ as modified by Irvine *et al.*³¹. All three inositol polyphosphates were checked for purity (>99%) and identified by iontophoresis³².

‡ Potassium salt prepared by mild alkaline hydrolysis³³ of phosphatidylinositol-4,5-bisphosphate, followed by oxidation with periodate and treatment with 1,1-dimethylhydrazine³⁴.

§ Ratio of Ins(1,4,5)P₃ to Ins(2,4,5)P₃ was 3:1.

|| Material that had been eluted from the same paper chromatogram on which InsP₃, Ins(1,4)P₂ and Ins(4,5)P₂ were separated but which did not contain inositol polyphosphates.

¶ These injections did not induce responses such as those shown in Fig. 1, but did produce small, often irreversible depolarizations that we attribute to either artefacts of the injection procedure or cell damage.

¶ Potassium salt; ** cyclohexylammonium salt; †† sodium salt.

those induced by InsP₃ injections but more often appeared to be the summation of discrete events (Fig. 1e,f). The responses induced by injections of inositol bisphosphate into a region of the cell not containing rhabdome were more delayed (Fig. 1e,f); the delay qualitatively depended on the apparent distance between the injection micropipette and the rhabdomic lobe of the cell. Responses induced by injections of inositol bisphosphate also apparently could be adapted by light, and vice versa.

It is difficult to estimate the intracellular concentration of inositol polyphosphates that is necessary to induce the physiological events that we describe, for two reasons. First, the amplitude of the injection-induced responses depends strongly on the location of the pipette within the cell, for both InsP₃ and InsP₂ compounds. Second, the details of diffusion from the tip of the injection pipette to the site or sites of action are unknown. For the same reasons, it is difficult to compare quantitatively the efficacy of different compounds or solutions of the same compounds at different concentrations.

The injection of DL-inositol 1-phosphate into the rhabdomic lobe had no noticeable effect on the cell (Table 2). For each of these cells, InsP₃ was injected from a second pipette placed

close to the pipette containing inositol-1-phosphate, to verify that the cell responded. Also, the injection of inositol hexaphosphate (phytic acid, InsP₆) had no noticeable effect on the cells (Table 2).

A stringent criterion for a putative messenger for excitation is that it must change the same specific ionic conductances as light. We tested InsP₃ according to this criterion by measuring the reversal voltages for both light-induced currents and InsP₃-induced currents; Fig. 2 shows that the reversal voltages were very similar. In five cells, the reversal voltages did not differ by more than 5 mV. Moreover, in one cell the reversal voltages for both light-induced currents and InsP₃-induced currents were measured at each of two concentrations of extracellular Na⁺ (425 mM and 106 mM, sucrose replacing NaCl)²³; in each solution, the reversal voltages differed by 4 mV. Thus, injection of InsP₃ into the rhabdomic lobe mimics several of the electrophysiological effects of a flash of light. We note that responses with the properties shown in Figs 1 and 2 are not induced by intracellular iontophoretic injections of calcium ions²⁴, or pressure injections of EGTA²⁵, ATP, cyclic AMP, GTP, cyclic GMP²⁶, or pH buffers²⁷.

Our biochemical and electrophysiological findings, reported here, are consistent with some criteria for the identification of InsP₃ as an authentic messenger for excitation in *Limulus* ventral photoreceptor cells. Our electrophysiological evidence also is consistent with an inositol bisphosphate or glycerol-1-phosphoinositol-4,5-bisphosphate being an excitatory messenger; the biochemistry necessary to test these compounds is incomplete at present. We do not yet know whether the magnitude or the kinetics of the light-induced changes in either the concentrations or the turnover rates of the intracellular inositol polyphosphates is consistent with the hypothesis; nor do we know whether the inositol bisphosphates have direct actions in the excitation process or cause a change in InsP₃ concentration, or have some other action on the cell's metabolism. Therefore, we tentatively suggest that light leads to the release of an inositol polyphosphate by the hydrolysis of PtdInsP₂ in the membranes of the photoreceptors; the inositol polyphosphate is diffusible within the cytosol and is one messenger in the cascade of excitation.

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Alternative sets of DNase I-hypersensitive sites characterize the various functional states of the chicken lysozyme gene

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The structural organization of chromatin is thought to determine the state of differentiation and activity of eukaryotic genes. Local interruptions of the regular nucleosomal array, the so-called DNase-hypersensitive sites, may indicate regions of the genome which play a critical part in regulation of differential gene activity^{1–4}. We present here two new observations on the chromatin structure of the chicken lysozyme gene, which strongly support a regulatory function for these sites. First, different sets of DNase I-hypersensitive sites have been found upstream from the promoter, depending on whether the gene is constitutively expressed (cultured macrophages) or in the steroid hormone-controlled state (oviduct). It seems, therefore, that diverse modes of regulation of the same gene are associated with discrete patterns of DNase I hypersensitivity. Second, one of the DNase I-hypersensitive sites in the oviduct chromatin disappears and reappears on steroid hormone withdrawal and secondary induction. These reversible changes in a narrow chromatin region reflect the transition from the potentially active to the active state of the lysozyme gene.

Nuclease hypersensitivity in chromatin involves short regions of approximately 50–400 base pairs that are roughly one order of magnitude more susceptible to nuclease cutting than is active chromatin as a whole^{1–5}. These regions are generally referred to as DNase-hypersensitive sites⁴ and have mostly been localized to the region near the 5' end of actively transcribed genes. Genes which are subject to complex regulatory mechanisms, such as developmentally controlled genes^{5–7} or genes under hormonal control^{7–9}, often display a more complex pattern of DNase hypersensitivity with multiple sites upstream and downstream from the gene.

The chicken lysozyme gene offers attractive features for studying the role of chromatin structure in activation and inactivation of a eukaryotic gene. In the chicken oviduct, synthesis of lysozyme mRNA is under the control of steroid hormones¹⁰ and can be reversibly induced in immature chickens by oestrogen administration or withdrawal¹¹. Expression of the same lysozyme gene in mature chicken macrophages is constitutive and transcription proceeds from the same promoter at a relatively low rate (ref. 12 and our unpublished S₁-mapping experiments). Liver cells, like most other cell types in the organism, do not express lysozyme mRNA (data not shown). It was conceivable that the chromatin might be organized differently depending on whether the cell in question is steroid hormone-responsive, nonresponsive or dormant.

We examined the chromatin structure of a 7.1-kilobase(kb) *EcoRI* fragment containing 4.6 kb of DNA sequences upstream

from the promoter of the chicken lysozyme gene, the first two exons and part of the second intron (see scheme in Fig. 3). Previously, we demonstrated that in the mature oviduct of egg-laying hens this region is marked by three hypersensitive sites referred to as HS3, HS4 and HS5 (ref. 8). As subsequent investigations have revealed a more complex pattern, we have re-named them according to their position with respect to the promoter of the gene (in kb) as HS–2.4, HS–1.9 and HS–0.1. Potentially active genes are often found to have hypersensitive sites independent of the transcription process *per se*^{3,4,7}. We therefore anticipated that only some of the sites upstream of the lysozyme gene might appear and disappear in correlation with steroid-mediated control of the transcriptional activity of the gene. To investigate this possibility, nuclei were prepared from the oviducts of immature chickens induced for 3 and 12 days with diethylstilboestrol, a synthetic oestrogen (primary induction). The small amount of oviduct cell mass in uninduced chickens precluded an assay for hypersensitive sites in birds with induction periods shorter than 3 days. Nuclei were mildly digested to varying extents by DNase I. DNA was then isolated and digested with *EcoRI* and analysed by the Southern blot technique using an indirect end-labelling procedure^{13,14}. After 3 days of exposure to oestrogen, the three sites HS–2.4, HS–1.9 and HS–0.1, characteristic of the mature oviduct of the egg-laying hen, are fully established and do not change during an additional 9-day period of primary induction (Fig. 1). If hormone administration is interrupted after 11 days, one of the hypersensitive sites (HS–1.9) disappears; on secondary induction with the oestrogen analogue it reappears. Thus, a close correlation can be demonstrated between the presence of the steroid hormone and the formation of the DNase I-hypersensitive site about 1.9 kb upstream of the transcriptional start site of the gene¹⁵. A similar observation was made in the chromatin of the vitellogenin gene⁷. The formation of a particular chromatin structure at a position far upstream appears to be associated with oestrogen activation of transcription. The site at –1.9 kb may be due to binding of the hormone–receptor complex, or to other DNA-binding proteins related to steroid action.

The hypersensitive site at –0.1 kb is only slightly less prominent 6 days after hormone withdrawal, indicating that the promoter region does not show considerable structural changes in response to the varying extents of oestrogenic induction and withdrawal.

To gain more information about functional aspects of the chromatin structure, we performed a second set of experiments in which the chromatin pattern was examined in various tissues of the egg-laying hen which do or do not express lysozyme (Fig. 2). The most striking result is that there are marked differences in the structural organization of chromatin between nuclei from two lysozyme-producing cell types. In mature macrophages, which constitutively express the gene, neither of the oviduct sites HS–1.9 or HS–2.4 is present; instead two new sites, HS–0.7 and HS–2.7, can be seen. This finding shows for the first time that different modes of transcriptional regulation of a eukaryotic gene correlate with different chromatin structures and supports the notion that the chromatin structure itself determines the functional state of a gene. The result further supports our finding that HS–1.9 is involved in steroid-dependent gene expression, as it is obviously not used for constitutive expression of the lysozyme gene. The formation of HS–1.9, however, cannot be the result of the mere presence of a functional oestrogen receptor complex because in liver, an oestrogen target organ⁷, this hypersensitive site is not formed (Fig. 2).

The hypersensitive site immediately upstream of the cap site, HS–0.1, is only present in cells which either express or have the potential to express the lysozyme gene (Figs 2 and 3). In tissues which do not express the lysozyme gene, the promoter region is buried within an inaccessible chromatin structure.

The hypersensitive site HS–2.4 is present in oviduct, liver, kidney, brain and in 5-day-old embryos, but is absent in macrophages and erythrocytes. There is no obvious function which correlates with this hypersensitive site. Based on its

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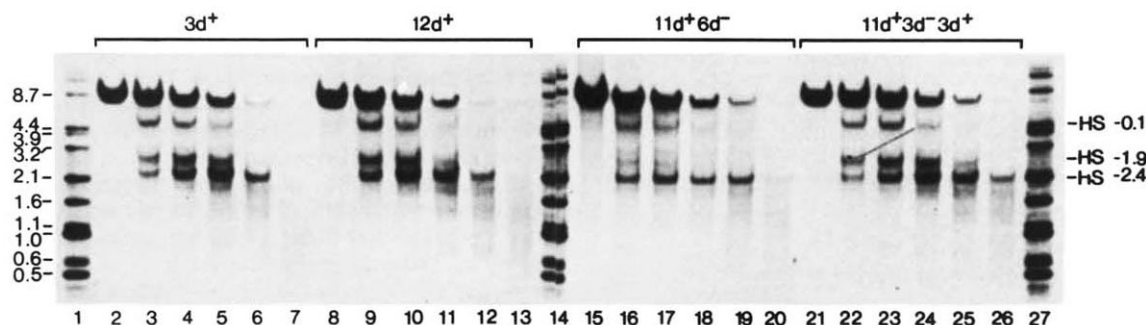


Fig. 1 Mapping of DNase I-hypersensitive sites in the 5' flanking region of the lysozyme gene in oviducts of diethylstilboestrol-induced, deinduced and reinduced immature chickens. Silicon tubes filled with crystalline diethylstilboestrol (DES) were implanted subcutaneously in 5-week-old female chickens¹⁷. Groups of chickens were either killed 3 and 12 days after implantation or hormone was withdrawn by removal of the tubes after 11 days. After 3 days of hormone withdrawal, one group of chickens was injected with 2 mg DES in sesame oil (secondary stimulation) while control chickens remained uninduced. Reinduced chickens and those in which hormone was withdrawn were killed 6 days after the start of the withdrawal period. Oviduct nuclei were prepared as described previously⁸ except that 2 mM EDTA and 0.5 mM EGTA were present in the isolation buffers. Digestion was with increasing concentrations of DNase I. Nuclear DNA was then isolated and completely digested with *Eco*RI. Southern blots were probed with radioactively labelled nick-translated plasmid DNA containing sequences between *Hind*III sites 4 and 5 (ref. 18) essentially as described previously⁸. The autoradiograph shows oviduct hypersensitive sites on the *Eco*RI fragment E4E5 (compare with scheme in Fig. 3) from 3-day-induced (3d⁺, tracks 2-7), 12-day-induced (12d⁺, tracks 8-13), 11-day-induced plus 6 days' hormone withdrawal (11d⁺6d⁻, tracks 15-20), and 11-day-induced/3 days' hormone withdrawal/3-day-reinduced (11d⁺3d⁻3d⁺, tracks 21-26) chickens. Tracks 1, 14 and 27 show DNA size markers, their length indicated in kb. The same results as seen in tracks 15-26 were obtained when oviduct chromatin was mapped from 12-day-induced followed by 3 or 6 days' hormone withdrawal, chickens (12d⁺3d⁻, 12d⁺6d⁻) and from 12-day-induced, 6 days' withdrawal, 3-day-reinduced chickens (12d⁺6d⁻3d⁺, data not shown). Hypersensitive sites (HS) were named according to their approximate location upstream of the cap site of the gene (in kb).

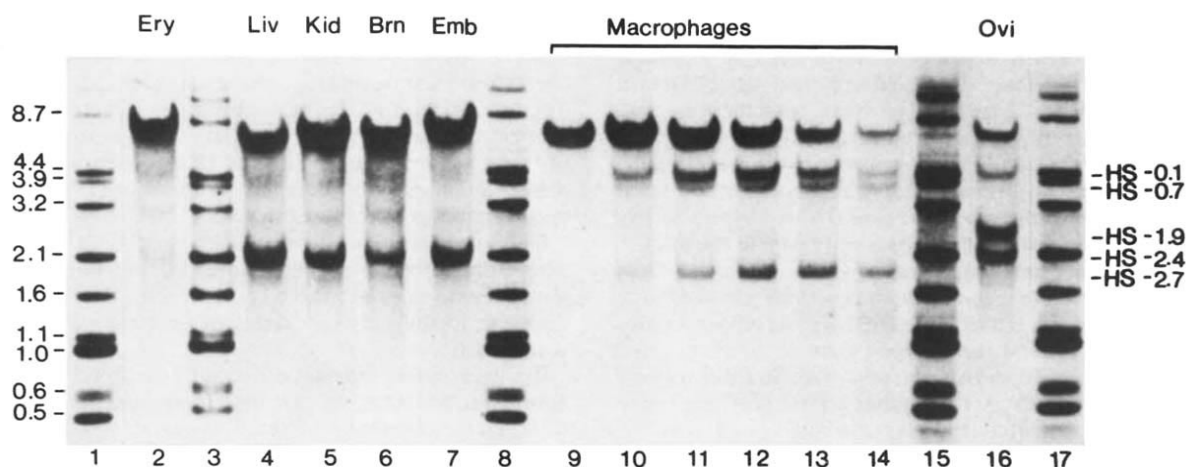


Fig. 2 Mapping of DNase I-hypersensitive sites in the 5' flanking region of the lysozyme gene in various lysozyme-producing and -nonproducing tissues of egg-laying hens and in tissue of total embryos. The autoradiograph shows hypersensitive sites mapped in nuclear chromatin of erythrocytes (track 2), liver (track 4), kidney (track 5), brain (track 6), mature oviduct (track 16) and whole 5-day-old embryos (track 7). Tracks 9-14 show the pattern obtained when nuclear chromatin of mature macrophages was mapped with increasing concentrations of DNase I. Mapping was done in all cases as described previously⁸. Tracks 1, 3, 8, 15 and 17 show DNA size markers (in kb). Macrophages were obtained from cultured white blood cells. Blood was collected by heart puncture and the buffy coat separated by centrifugation at 800g for 10 min through a Ficoll solution (lymphocyte separation medium, density 1.077; Flow) and seeded at 1×10^8 cells per 150 cm² flask in growth medium (MEM-Iscove; 8% fetal calf serum, 2% chicken serum; Boehringer). The adherent cell fraction was scraped off after 5 days and lysed by Dounce homogenization at 2×10^7 cells ml⁻¹ in homogenization buffer⁸ modified as described in Fig. 1 legend.

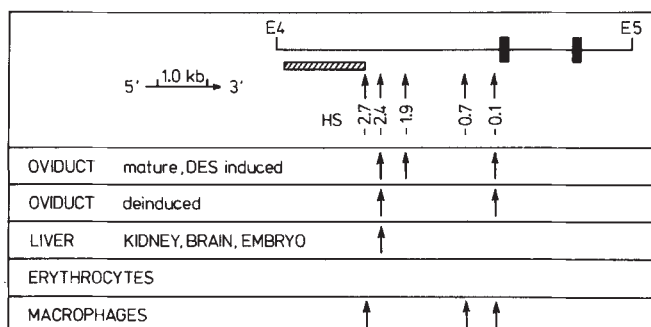


Fig. 3 Scheme showing the positions of DNase I-hypersensitive sites in the 5' flanking region of the lysozyme gene in various chicken tissues. E4 and E5 denote relevant *Eco*RI sites 4.6 kb upstream and 2.5 kb downstream of the promoter of the gene¹⁸. Solid boxes represent exon regions and the hatched bar shows the position of the subcloned *Hind*III fragment (H4H5 in ref. 18) used as radioactive probe in the procedure for mapping DNase I-hypersensitive sites. Arrows indicate the positions of hypersensitive sites as shown in Figs 1 and 2.

absence or presence in the various tissues, we propose that HS -2.4 has a role in suppressing lysozyme gene activity. Such a function should not be present in macrophages which express the lysozyme gene constitutively, but would be required in the oviduct, in which transcription occurs in a reversible fashion depending on the hormonal state. In the latter case, the steroid-inducible site HS -1.9 would override the presumed function of HS -2.4.

Implicit in our interpretation is the assumption that the upstream hypersensitive sites are involved in the control of the lysozyme gene itself rather than being indicative of a yet unidentified adjacent transcription unit. As *in vitro* transcription analyses of the respective DNA sequences revealed transcripts starting only at the lysozyme promoter (M. Theisen, unpublished), we feel this bias to be tenable.

DNase I-hypersensitive sites are believed to be short nucleosome-free regions which mark sites where regulatory proteins gain access to specific DNA sequences¹⁶. Evidence is accumulating that these structures not only signal the actual transcriptional state of a gene but also the state of differentiation. The correct formation of these structures during differentiation seems to be crucial for differential expression. It remains to be shown how chromatin structural changes observed kilobases away from the transcriptional initiation point can determine the state of differentiation and activity of a eukaryotic gene.

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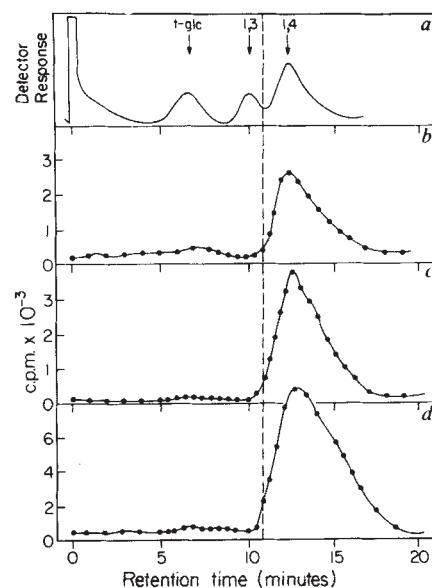


Fig. 1 Gas liquid chromatography of permethylated derivatives of the ^{14}C -labelled-24% KOH-insoluble product formed from UDP- ^{14}C glucose. *a*, Recorder tracing of derivatives obtained from a mixture of laminarin and cellobiose. Peaks were identified by comparison with derivatives of the individual compounds (1,3 = permethylated derivative of laminarin; 1,4 = permethylated derivative of cellobiose; t-glc = terminal glucose). The lower three curves represent elution profiles of radioactivity of permethylated derivatives from products formed in standard assay mixtures, 5 μM (*b*), 500 μM (*c*) and 1 mM UDP- ^{14}C glucose (*d*).

In all the studies described here, data are presented only for the radioactive 24% KOH-insoluble glucan product formed from UDP- ^{14}C glucose, which has been characterized as 1,4- β -D-glucan by the following criteria: (1) Total acid hydrolysis converted >90% of the radioactivity to ^{14}C -glucose. (2) The product was more than 95% digested by either highly purified *Trichoderma reesei* exocellobiohydrolase or by highly purified *Trichoderma* endo-1,4- β -glucanase (on chromatography of the exocellobiohydrolase digest, on Whatman No. 4 paper in *n*-propanol/ethyl acetate/water, 7:1:2, v/v, >80% of the soluble radioactivity co-chromatographed with a cellobiose standard and the remainder was found at the origin and in glucose). (3) Methylation analysis² of the products formed either from 5 μM , 500 μM or 1 mM UDP-glucose, yielded exclusively 1,4,5-tri-O-methyl-2,3,5-tri-O-methyl- ^{14}C glucitol, the derivative expected for 1,4-linked glucosyl moieties (Fig. 1). (4) periodate oxidation⁴, followed by borohydride reduction, complete acid hydrolysis and paper chromatography yielded primarily erythritol (indicative of 1,4-linkages), a trace of glycerol and some material which remained at the origin. In no case could a significant peak of glucose (indicative of 1,3-linkages) be detected. The small peak of glycerol detected amounted to 1-3% of the labelling of the major erythritol peak. Assuming that this glycerol arises from terminal glucose residues, it indicates that the enzyme is producing long chains and is not a chain-terminating enzyme adding just single glucose residues to pre-existing polysaccharides.

The procedure for preparing the enzyme is summarized in Fig. 2. If cells were ruptured in an extracting medium containing 20% polyethylene glycol 4000 (PEG), the membranes and all associated glucan synthase activity (when assayed under standard assay conditions, see below) pelleted at relatively low centrifugal forces (12,000g for 10 min). In contrast, in the absence of PEG, higher centrifugal forces (150,000g for 60 min) were needed to sediment membranes and the enzyme activity. A similar effect of PEG has been observed with other plant glucan synthases¹ and with *A. xylinum* 1,4- β -D-glucan synthase²,

High rates of *in vitro* synthesis of 1,4- β -D-glucan in cell-free preparations from *Phaseolus aureus*

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Progress in the study of biosynthesis of cellulose (1,4- β -D-glucan) has been persistently hampered by the low rates of *in vitro* synthesis obtained using cell-free preparations derived from plant or bacterial cells capable of ample cellulose synthesis *in vivo*¹. We recently reported the achievement of high rates of synthesis of 1,4- β -D-glucan from UDP-glucose, using membrane preparations obtained from the bacterium *Acetobacter xylinum*. The key to the success lay in the discovery of a complex GTP- Ca^{2+} -protein factor-mediated regulatory system for the *A. xylinum* UDP-glucose- β -D-glucosyltransferase (cellulose synthase)^{2,3}. We now describe conditions found for an extremely efficient transfer of glucose from UDP-glucose to a cellulosic 1,4- β -D-glucan product using enzyme preparations derived from a higher plant, seedlings of mung beans (*Phaseolus aureus*). The rates of enzyme activity achieved approach those of cellulose synthesis observed *in vivo*.

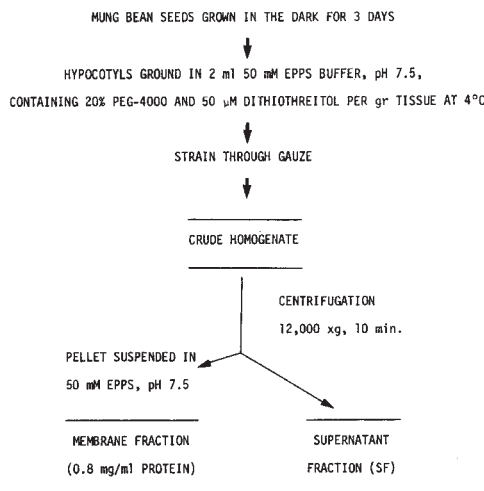


Fig. 2 Protocol for preparation of enzyme.

and may be attributed to the known effect of PEG in promoting membrane fusion and protein coagulation⁵. PEG treatment also enhanced 4–5-fold, the rate of product formation by the membrane preparation. This effect could involve protection of the enzyme during cell breakage and/or promotion of interaction of enzyme subunits. Activity was retained in PEG preparations for two months when these were frozen in liquid nitrogen and kept at -70°C .

Standard assays performed at 20°C contained in a final volume of 0.2 ml: 50 mM *N*-(2-hydroxyethyl)-piperazine-*N'*-3-propanesulphonic acid buffer, pH 7.5, 5 μ M UDP-[^{14}C]glucose (320 c.p.m. pmol^{-1}), 6 mM MgCl_2 , 4 mM CaCl_2 , 5 mM cellobiose and enzyme. Reactions were terminated by addition of 2 ml 24% KOH. Approximately 20 mg of carrier cellulose was added, and the mixture incubated for 12 h at 40°C (ref. 6). The mixtures were then filtered onto Whatman GF/A glass fibre filters, and washed consecutively with water and methanol. The filters containing alkali-insoluble product were dried and counted. Under these conditions, reactions were linear with time for up to 30 min and proportional to enzyme concentration up to 100 μg protein. At pH 8.6, activity was considerably lower and linearity with time ceased after 2–5 min.

Requirements for the reaction are shown in Table 1. The enzyme has an absolute requirement for both Mg^{2+} and Ca^{2+} . Whereas the dependence on Mg^{2+} was demonstrable with regular enzyme preparations, the effect of Ca^{2+} was much more pronounced when EGTA was included in the medium used for extracting the enzyme. The particulate membrane fraction sedimenting from centrifuged crude homogenates exhibits low activity. Activity is restored on re-addition of the supernatant

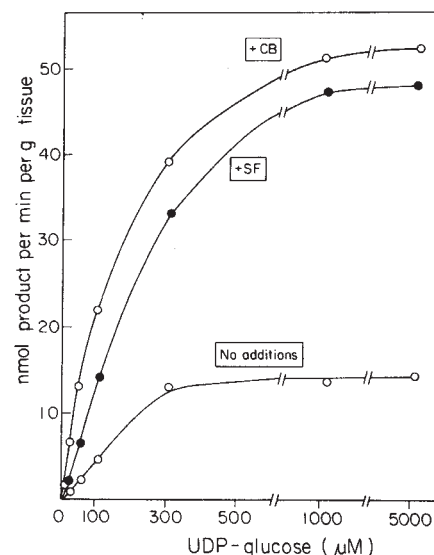


Fig. 3 The effect of varying the concentration of UDP-glucose on enzyme activity. UDP-glucose concentrations were as indicated, and membrane fraction derived from 10 mg fresh weight of tissue (0.04 mg protein) was used per assay. Additions indicated were: 5 mM cellobiose (+CB) and 25 μ l of supernatant fraction (+SF).

fraction (SF). The supernatant alone was found to contain no synthase activity (data not shown). Product formation is markedly increased by the addition of cellobiose to the incubation mixture. However, the extent of this effect is dependent on the enzyme preparation. Whereas addition of cellobiose to centrifuged fractions results in a tenfold increase in activity, in the presence of the SF only a twofold increase is observed. Activity of the particulate fraction increases with increasing cellobiose concentrations and with varying levels of SF. Half-maximal stimulations are achieved with 0.75 mM cellobiose and 1.5 μ l of SF (an amount of supernatant derived from 0.8 mg fresh weight of tissue). The enzyme displays normal Michaelis-Menten kinetics with respect to UDP-glucose (Fig. 3). The presence of saturating amounts of cellobiose or SF does not alter the apparent K_m for the substrate (100 μ M) but the $V_{\max}$ is enhanced about fivefold (compare refs 7 and 8). Preliminary characterization of the stimulatory factor in SF, based on stability studies, indicate that it is an organic dialysable, alkali-labile compound which is absorbed by charcoal. It is resistant to heat and acid treatment (0.1 M HCl, 10 min at 100°C) and to digestion by exocellulohydrolase, endo-1,4- β -glucanase, β -glucosidase, crude cellulase and alkaline and acid phosphatases. Unlike the 1,4- β -glucan synthase of *A. xylinum*², the plant enzyme has not been found to respond to the addition of GTP. The SF, however, strongly interferes with GTP activation of the bacterial enzyme.

Incorporation of labelled glucose from UDP-[^{14}C]glucose into the cellulosic product was neither stimulated nor inhibited by the addition of an up to 100-fold excess of unlabelled UDP-xylose or GDP-glucose (compare refs 6, 9–11). Coumarin and dichlorobenzonitrile, specific inhibitors of plant *in vivo* cellulose synthesis¹² strongly inhibit our enzyme (Table 1). Note that these compounds inhibit GTP activation of the *A. xylinum* 1,4- β -glucan synthase (Y. Aloni and M.B., unpublished results).

Because the reaction product is clearly a 1,4- β -D-glucan, we presume that we are dealing with a true cellulose synthase; however, lacking information on the crystalline structure and microfibrillar nature of the product, we referred to it at present simply as a 1,4- β -D-glucan synthase. The rates of synthesis of 1,4- β -glucan *in vitro* reported here far exceed those previously reported^{13–17}, and approach those of cellulose synthesis observed *in vivo* during primary cell wall formation (12–30 nmol per min per g fresh weight of tissue)^{15,17,18}. Thus, the long awaited goal

Table 1 Factors affecting UDP-glucose:1,4- β -D-glucan synthase activity

Incubation mixture	Activity (pmol product per min per g tissue)
Complete system	1,700
Minus Mg^{2+}	50
Minus Ca^{2+}	40
Minus SF minus cellobiose	170
Minus cellobiose	850
Minus SF	1,700
Plus DCB (6 μ M)	120
Plus coumarin (2 mM)	40

The complete system contains: crude homogenate derived from 10 mg of tissue in the presence of 5 mM EGTA; 6 mM MgCl_2 ; 4 mM CaCl_2 ; 5 mM cellobiose; and 5 μ M UDP-[^{14}C]glucose. DCB = 2,6-dichlorobenzonitrile.

of obtaining from a plant source a highly active system for 1,4- β -D-glucan synthesis *in vitro* has been accomplished. This system with its interesting properties, especially in relation to the bacterial synthase system, should be of great value for exploring further the details of the mechanism and regulation of cellulose synthesis.

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6 Å-Resolution X-ray structure of a variable surface glycoprotein from *Trypanosoma brucei*

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The variable surface glycoprotein (VSG) is the predominant component of the surface coat of the African trypanosome¹. The expression of antigenically distinct VSGs on minor populations during infection allows the parasite to escape the host immune response^{2,3}. Purification of the protein is facilitated by the enzymatic release of a soluble form of VSG (sVSG) which occurs on cell lysis⁴. The soluble form is a dimer with an approximate molecular weight of 120,000–130,000⁵. Partial proteolysis of sVSG⁶ reveals a protease-sensitive link between an amino-terminal domain which comprises about two-thirds of the molecule, and a C-terminal domain which contains the membrane attachment site⁷. We have obtained crystals suitable for high-resolution structural analysis from preparations of three sVSG: MITat 1.2, ILTat 1.25 and ILTat 1.22. The crystal structure of the dimer of the MITat 1.2 amino-terminal domain has been solved to 6 Å resolution. We report here that the dimer is an unusual 90 Å rod-like molecule composed of a helical bundle of at least four 80 Å-long α -helices.

sVSG was purified from *Trypanosoma brucei* as described previously¹. MITat 1.2 was crystallized by vapour diffusion of a 40 mg ml⁻¹ solution in 50 mM Tris-HCl/0.1% sodium azide, pH 7.5 against 9% polyethylene glycol (PEG) 8000/10% ethylene glycol in the same buffer. Tetragonal bipyramidal crystals, which diffract to 2.9 Å, appear within a week and grow to a maximum dimension of 1 mm. The space group is P4₁2₁2, with $a = 96.3$, $c = 111.3$ Å; the asymmetric unit contains one monomer of the amino-terminal domain. Details of the crystallization of ILTat 1.22 and ILTat 1.25 will be published elsewhere.

Protein recovered from MITat 1.2 crystals has an apparent molecular weight (MW) of 43,000 (43 K) on SDS-polyacrylamide gel electrophoresis (PAGE), co-migrating with the amino-terminal domain identified by Johnson and Cross⁶ (Fig. 1). The amino-terminal sequence of the recovered protein is Ala-Ala-Glu-Lys, consistent with the previously published N-terminal sequence⁶. By using 'immuno-blotting', (data not shown) we have

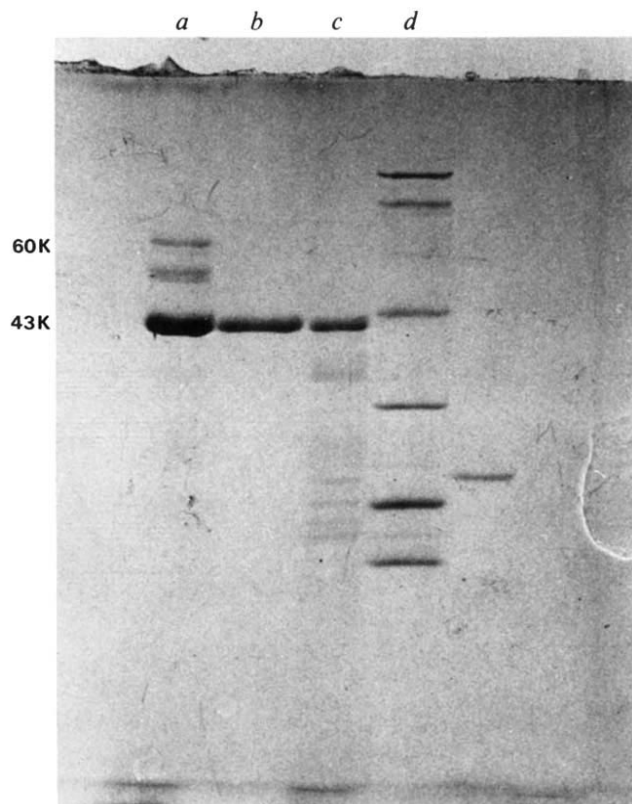


Fig. 1 Protein recovered from MITat 1.2 crystals migrates with an apparent MW of 43,000 (43K) on a 12% SDS polyacrylamide gel. *a*, 5 μ g MITat 1.2 from a solution used for crystallization. The intact polypeptide migrates with an apparent MW of 60,000 (60K); the fragment at 43K corresponds to protein recovered from the crystal. *b*, Two large (0.5, 1 mm) crystals were washed in 2 ml 12% PEG, 10 mM TrisHCl, 0.1% azide, pH 7.5, and dissolved in a capillary with 30 μ l 10 mM TrisHCl pH 7.5. The dissolved protein was boiled for 3 min after addition of 90 μ l sample buffer; an aliquot of 20 μ l was loaded on the gel. *c*, 5 μ g MITat 1.2 was digested with trypsin, using the conditions of Johnson and Cross⁶; the digestion was stopped by adding excess phenylmethanesulphonyl fluoride and boiling after addition of sample buffer. The amino-terminal protease-resistant fragment co-migrates with the crystallized protein. *d*, Bio-Rad molecular weight standards: 92.5K, 66.2K, 45.0K, 31.0K, 21.5K and 14.5K.

demonstrated that the protein recovered from the crystal does not contain the C-terminal 'cross-reacting determinant'⁹ of the intact VSG. Thus, it seems that adventitious proteolysis from a contaminating protease releases an N-terminal crystallizable domain. On gel permeation HPLC (data not shown) protein recovered from MITat 1.2 crystals elutes with an approximate molecular weight of 80,000, clearly distinguishable from the intact native dimer at around 120,000, suggesting that the crystalline protein is a dimer of two 43K domains.

A mercury derivative was obtained after a 1-day soak in a 12% PEG solution (otherwise identical to the mother liquor) saturated with K₂HgI₄. Data from one native crystal and one Hg derivative crystal were collected by diffractometer. Data were corrected for Lorentz polarization effects and radiation damage, and an empirical absorption correction was applied¹⁰. Friedel equivalents were scaled together in blocks of reciprocal space¹¹. Data collection statistics are summarized in Table 1.

Harker sections of isomorphous and anomalous difference Pattersons showed identical peaks, and were interpretable in terms of a single Hg site at 0.20, 0.19, 0.50. After 10 cycles of refinement of the heavy-atom parameters, including the anomalous scattering data, 1,211 reflections (89% of the 15–6 Å shell) were phased with a mean figure of merit of 0.72 (Table 1). A difference Fourier showed no additional Hg sites.

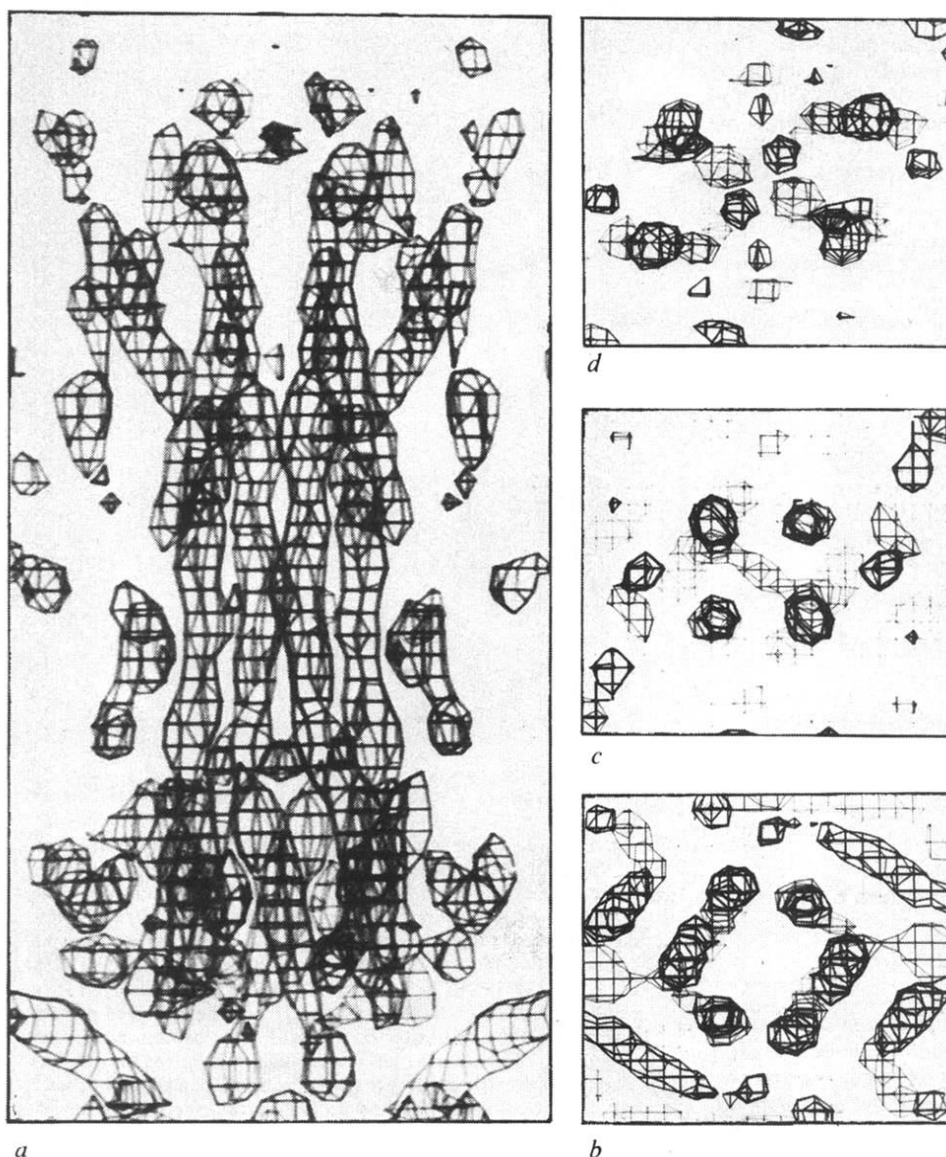


Fig. 2 *a*, View of the electron density map perpendicular to the dimer axis, which is vertical in the figure. The map was contoured two standard deviations above mean density and displayed on an Evans and Sutherland PS300 using the FRODO molecular graphics program by J. W. Pflugrath, M. A. Saper, B. Bush and A. Jones. At the bottom is the 6-fold bundle extending about 25 Å along the dimer axis. At the top, the density diverges, forming a 'two-headed' structure. (Symmetry-related density is seen in the lower corners.) *b–d*, Regions of the map shown in *a*, viewed along the dimer 2-fold axis. *b*, Approximately 20 Å from the 'bottom'. The 6-fold bundle of electron-dense rods is apparent. The distance between rods of density is ~10 Å. Unconnected density occurs adjacent to the bundle. *c*, Approximately 50 Å from the 'bottom'. The 4-fold bundle is the continuation of density which begins in *b*. *d*, Approximately 80 Å from the 'bottom'. Density from each monomer diverges and appears to form two distinct bundles.

The native electron density map calculated using the 'best' phases¹² shows that the unit cell contains four dimers packed along the crystallographic 2-fold axes. At this resolution, rods of high electron density ~10 Å apart are clearly resolved, forming a bundle which extends about 80 Å. We interpret this as an α -helical 'core' of the dimeric molecule.

The dimer is elongated, with approximate dimensions of 40 × 40 × 90 Å (Fig. 2*a*). When viewed down the dimer axis, the helical bundle is apparent (Fig. 2*b–d*). The connectivity of two α -helices in each monomer can be traced for about 80 Å. At one end (bottom of Fig. 2*a*) density connects the two helices, which form a 25 Å-long left-handed 6-fold bundle with another, unconnected, helical segment (Fig. 2*b*). The two helices from each subunit extend past this region to form a fairly straight four- α -helical bundle which extends about 30 Å (Fig. 2*c*). A third rod of high electron density begins adjacent to this structure. The strands then diverge to form two left-twisted structures, about 30 Å long, each of which clearly involves three helices, and possibly other adjacent density, at the 'top' of each subunit (Fig. 2*d*).

We estimate, by crude fitting of polyalanine α -helices, that about 175 residues are accounted for by the well defined helical regions of the low-resolution map. This is about 50% of the expected length of the polypeptide based on its migration on SDS-PAGE, and its consistent with circular dichroism results (M.J.T. and T. Bayley, unpublished data). We have not yet

established the connectivity of additional density which occurs at the end of the dimer and around the helical bundle.

The long rod-like α -helical core of the VSG is similar, in principle, to the trimeric α -helical coiled-coil in the stem region of the influenza virus haemagglutinin (HA)¹³. The occurrence of helical motifs in other surface glycoproteins has been discussed¹⁴. The connectivity observed here between the two long helices in the MITat 1.2 amino-terminal domain implies a helical region extending about 120 residues with only one break. If such a structure were common to VSGs of diverse sequences, it might be revealed by analysis of secondary structure predictions. Alternatively, this feature might readily be recognized in the low-resolution structure of a second VSG. Although interesting sequence homology among VSGs has been observed in the C-terminal region^{15–18}, the extent of structural homology is unknown¹⁹.

Recent work²⁰ has shown that the N-terminal domain of another VSG, MITat 1.6, binds most monoclonal antibodies against the intact VSG molecule; that only a subset of the monoclonal antibodies which bind to isolated intact VSG will interact with the VSG when it is in the trypanosome surface coat; and that the subset of monoclonal antibodies which bind to the trypanosome coat also bind to the N-terminal domain. These results suggest that the N-terminal domain contains the regions of the VSG which are responsible for the antigenic variation recognized by antisera against live trypanosomes. Thus

Table 1 6 Å data collection and phase refinement for MITat 1.2 VSG

	Native	Hg
Cell dimensions (Å)	96.3, 111.2	96.4, 111.6
No. of reflections collected	2,781	2,812
Friedel <i>R</i> factor		
Centric	No. of pairs 1.7% 381	No. of pairs 1.4% 387
Acentric	2.2% 950	3.9% 979
No. of reflections phased	1,211	
Mean figure of merit	0.72	
Kraut <i>R</i>	7.39	
$\langle Fh \rangle / \langle E \rangle$	2.97	
Friedel <i>R</i> factor:	$\text{sum}(I^+ - I^-) / \text{sum}(I^+ + I^-)$	
Kraut <i>R</i> :	$\frac{\text{sum}[Fph(\text{obs}) - Fph(\text{calc})]}{\text{sum}[Fph(\text{obs})]} \times 100\%$	
	<i>Fph</i> = derivative structure factor	
$\langle Fh \rangle / \langle E \rangle$:	$\frac{\text{r.m.s. heavy-atom structure factor}}{\text{r.m.s. lack of closure error}}$	

Before data collection, MITat 1.2 crystals were transferred to a solution containing 12% PEG, 10% ethylene glycol, 0.1% azide, 50 mM Tris HCl pH 7.5. Data were collected using a Wyckoff step scan²¹ on a Syntex P3/F diffractometer equipped with a 50-cm helium-filled extension tube. About 3,000 reflections, including Friedel pairs, were collected in the 15–6 Å shell. Four reference reflections were collected every hundred reflections; the mean decay was 26% (native) and 21% (Hg) over the 3 days of data collection. The length of the *c* axis was observed to change by 1% during the data collection. Difference Patterson maps were calculated using coefficients ($Fph - Fp$)*²² and ($Kemp^{*2/4}(Fph^+ - Fph^-)$)*²², where $Kemp^{22}$ was 6.43. The heavy-atom position and occupancy were refined using the program PHARE3, which alternates cycles of parameter refinement and phase calculation²³. The anomalous scattering information was included in the refinement²⁴, and was used to distinguish the correct space group, P4₁2₁2, from its enantiomer²⁵. B was held at 25 Å². The final mercury position, after 10 cycles, was *x/a* = 0.22, *y/b* = 0.19, *z/c* = 0.50 with occupancy 0.58. The iodine atoms were not included in the refinement.

the three-dimensional structure of the N-terminal domain should contribute to establishing which parts of the sequence are implicated in the generation of antigenically distinct trypanosomes and to determining the structure of the variable epitope(s).

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α -Helical coiled-coil structures of *Trypanosoma brucei* variable surface glycoproteins

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We have used electron microscopy to examine purified intact variable surface glycoproteins (VSGs) from clones derived from two distinct stocks of *Trypanosoma brucei*. The VSG molecule from MITat 1.2 has a large elongated domain consistent with the shape of the dimeric N-terminal domain determined by X-ray analysis (see preceding paper¹), and a heretofore unseen short, thin fibrous tail presumed to be the C-terminal domain. Electron microscopy on DiTat 1.3, however, indicates a morphology quite distinct from that of MITat 1.2. Analysis of four VSG amino acid sequences reveals 7-fold periodicities (heptad repeats) which indicate that α -helical coiled-coil secondary structure elements occur in all of these VSGs, consistent with the observation of helical bundles in one VSG¹. These results suggest the possibility that VSG antigenic diversity may be related to a diversity in length and disposition of α -helical bundles and coiled-coil domains.

We have determined the overall morphology of certain VSG molecules in the electron microscope by rotary shadowing with platinum. The first observations were carried out on DiTat 1.3. Although the material obtained from lyophilized preparations shows considerable heterogeneity, many molecules in the field display a distinctive shape; these are ~180 Å long and consist of a globular 'head' region (~60 Å diameter) attached to a narrower 'stem' about 100 Å long and 30 Å thick that can often be seen to terminate in a small globular domain (Fig. 1). A variety of smaller fragments related to this form may also be seen (Fig. 1). SDS gels of this material indicate that most of the sample has an apparent molecular weight of 64,000 (Fig. 2). Shadowing of mixtures of VSG and tropomyosin (MW 66,000) suggests that the molecules we have observed are VSG dimers (MW 130,000). Digestion with trypsin (data not shown) indicates that the large globular head region contains the N-terminus.

We then examined intact MITat 1.2 VSG molecules whose N-terminal dimer structure has recently been solved¹. This VSG species appears to have a markedly different morphology: particles with a major domain about 100 Å × 70 Å are observed. No clear 'stem' region is apparent, but a short tail can sometimes be seen (Fig. 3). Dissolved crystals of the dimer of the N-terminal domain show similar compact particles that lack tail regions (Fig. 3). These images agree well with the results of the X-ray structure determination, considering that particle flattening often occurs in rotary-shadowed preparations. The presence of the slender tail regions may account for difficulties in crystallizing the intact molecules¹.

Sequence information is not yet available for either of the VSG molecules that we have examined, but we have carried out conformational analyses of the published sequences of four other VSGs from the two C-terminal subsets that have been distinguished^{2,3} (including VSG 117 (MITat 1.4) (ref. 4), AnTat 1.1 (ref. 5), ILTat 1.1 (ref. 6), and ILTat 1.4 (ref. 7)). Detailed findings will be reported elsewhere, but the major conclusions are summarized here. Secondary structure analyses were based on modifications of the procedures of Chou and Fasman⁸, Robson and Suzuki⁹ and Garnier *et al.*¹⁰. On this basis, some

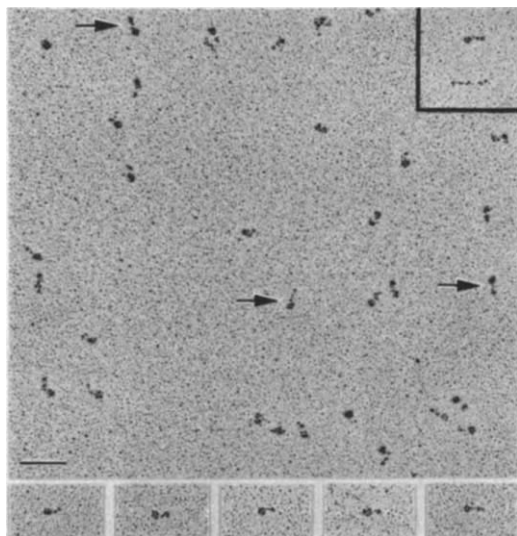


Fig. 1 Electron micrographs of rotary-shadowed VSG (*T. brucei brucei* clone T45R10, antigenic type DiTat 1.3). The molecules are ~ 180 Å long and show three distinct domains: a large 'globular' head region about 60 Å in diameter; a 'stem' ~ 100 Å long and 30 Å wide; and a smaller globular region ~ 40 Å in diameter. Molecules are also observed that lack the small globular region, thus appearing to have a 'tail' about 140 Å long. The field and selected molecules illustrate both types of image. Inset, a cardiac tropomyosin molecule (MW 66,000; dimensions 400 Å $\times$ 20 Å) shadowed together with VSG. The VSG molecules probably correspond to dimers. Replicas were prepared by rotary shadowing with platinum¹⁷. Measured dimensions may vary by ± 20 Å depending on the thickness of the platinum deposit. Scale bar, 500 Å.

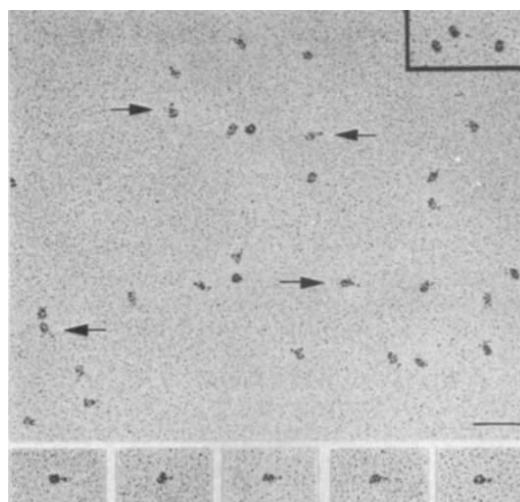


Fig. 3 Electron micrographs of rotary-shadowed VSG (*T. brucei brucei*, antigenic type MITat 1.2). The dimeric molecules display a large 'globular' domain about 110 Å $\times$ 70 Å and often a shorter (~ 50 – 100 Å) 'tail' region. Occasionally, the tail terminates in a very small globular domain. Inset, the N-terminal dimer portion of the molecule from redissolved crystals of this domain prepared as described by Freymann *et al.*¹. Experimental details of shadowing as for Fig. 1. Scale bar, 500 Å.

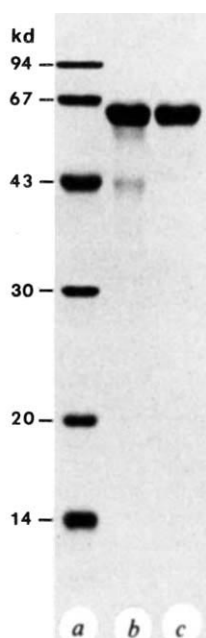


Fig. 2 Intact DiTat 1.3 monomer has an apparent molecular weight of $\sim 64,000$ by SDS-polyacrylamide gel electrophoresis. Lanes *b* and *c* show two different VSG samples, both purified from *T. brucei brucei* clone T45R10, antigenic type DiTat 1.3. The material was prepared by the method of Cross¹⁸ and Roelants *et al.*¹⁹ with affinity chromatography on concanavalin A-Sepharose as the final purification step, followed by lyophilization. Lane *a* shows a mixture of low-molecular weight markers (phosphorylase *b*, albumin, ovalbumin, carbonic anhydrase, trypsin inhibitor and α -lactalbumin; supplied by Pharmacia Fine Chemicals). Laemmli SDS-polyacrylamide gel (12.5%) electrophoresis.

50% of the VSG conformation may be α -helical. We also carried out a search for quasi-repeating heptads of the form $(a-b-c-d-e-f-g)_n$ where *a* and *d* are apolar residues^{11,12} that allow an interlocking of α -helices, as envisaged by Crick, to form a coiled-coil structure¹³. Our analysis reveals that the N-terminal domains show extended 7-fold periodicity indicative of a high proportion of α -helical coiled-coil structure which differs, however, in extent and disposition between the subsets. Examples of such 7-fold repeats in two subset I VSG sequences are shown in Table 1. The extent of the regions exhibiting the heptad repeat is such that *n* commonly lies between 2 and 7, which is much less than that found for tropomyosin (where *n* = 40), but comparable to that in the rod segments of fibrinogen¹¹. It should be stressed that 'broken heptads' of the type found here (with discontinuities in regularity) are compatible both with a long stretch of coiled-coil or with shorter stretches of α -helices interlocking in a more compact structure (such as a 4- α -helical motif¹⁴). For example, the coiled-coils could form a compact region followed by an extended stem (perhaps as in DiTat 1.3) or a more elongated bundle (as in MITat 1.2). The heptads found in the sequences have a markedly higher content of hydrophobic residues in positions *b*, *c*, *e*, *f* and *g* than those of α -helical coiled-coil muscle proteins (Table 1). This feature may be related to the characteristic dimerization of these molecules (see also 1). Although the C-terminal domains of VSG molecules may be morphologically distinct in different species (as in DiTat 1.3 and MITat 1.2), a highly charged 25-residue segment appears to be conserved in both subsets and is likely to have a membrane-binding role. Our results on α -helix content are in general agreement with the recent study of Lalor *et al.*¹⁵; we believe, however, that consideration of the heptad regularity is a critical aspect of the sequence analysis and one which has important structural and functional implications.

At present only parts of the puzzle are available to establish strict morphology/sequence/function relations in the VSGs. Nevertheless, our results, taken together with the recent X-ray crystallographic studies of Freymann *et al.*¹, suggest some design principles for these surface glycoproteins. As described by Cohen and Phillips¹⁶, the α -helical coiled-coil is a fundamental feature of the structure. The length and disposition of α -helices in the exposed N-terminal variable region may differ, however, in different species of VSG (see also ref. 15). It is also possible that the broken heptad repeats in the sequences provide a device for generating structural diversity. Thus, the α -helical segments

Table 1 Heptad regularities in α -helical regions of the N-terminal domains of two subset I VSGs

Residue	a	b	c	d	e	f	g
VSG 117 (MITat 1.4)							
7 heptads	Leu	Cys	Gly	Leu	Ala	Ala	Thr
	Val	Arg	Lys	Val	Ala	Gly	Gly
	Val	Leu	Thr	Lys	Leu	Lys	Ser
	His	Ile	Ser	Tyr	Arg	Lys	Lys
	Leu	Glu	Glu	Met	Glu	Thr	Lys
	Leu	Arg	Ile	Tyr	Ala	Leu	Lys
	Gly	Asp	Gly	Val	Gly	Glu	Gln (Lys Ser)
2½ heptads	Ala	Glu	Ile	Leu	Ala	Thr	Thr
	Ala	Ala	Leu	Met	Arg	Gln	Lys
	Ala	Leu	Thr	(Pro	Glu	Glu	Ala Asn)
3½ heptads	Leu	Lys	Thr	Ala	Leu	Lys	Ala
	Ala	Gly	Phe	Ala	Gly	Glu	Gly
	Ala	Ala	Ala	Val	Ser	Ser	Tyr
	Leu	Met	Thr	Leu	(Gly	Thr	Leu
	Thr	Thr	Ser	Gly	Ser	Ala	His Cys)
AnTat 1.1							
6 heptads	Ala	Cys	Thr	Tyr	Ser	Lys	Thr
	Leu	Lys	Arg	Gln	Ala	Ala	Asn
	Ala	Ala	Gln	Thr	Leu	Gln	Arg
	Ala	Ser	Ser	Ala	Ala	Lys	Gln
	Ser	Arg	Gln	Ala	Gln	Gln	Leu
	Ala	Ala	Leu	Ala	Leu	Ala	Lys
2 heptads	Leu	(Pro	Asp)				
	Tyr	Lys	Glu	Ala	Ala	Ala	Thr
5 heptads	Leu	Leu	Ile	Tyr	Ala	Thr	His (Lys)
	Ile	Gln	Asp	Ala	Gln	Ala	Ser
	Ile	Glu	Asn	Trp	Thr	Gly	Glu
	Asn	Thr	Lys	Leu	Val	Gly	Gln
	Ala	Met	Tyr	Ser	Ser	Gly	Arg
	Ile	Asp	Glu	Leu	Met	Leu	Leu
	Leu	Glu	(Gly	His	Arg	Glu	Asp
	Gly	Ala	Asn	Gly	Gln	Asp	Lys Thr Cys)

Apolar residues in positions *a* and *d* permit the adoption of a coiled-coil structure. Residues in parentheses introduce a discontinuity in the heptad repeat, represented by a space in the sequence. The upper sequence is from VSG 117 (MITat 1.4) between Cys 14 and Cys 121. The lower sequence is from AnTat 1.1 between Cys 45 and Cys 154. The sequences of AnTat 1.10 and 1.1B give similar results. Subset II VSGs appear to be somewhat different: broken heptad runs occur farther along in the sequence—for example, between residues 237 and 339 in ILTat 1.1 and 151 and 277 in ILTat 1.4.

may adopt a variety of closely related forms, from extended to compact, that depend as well on the constraints imposed by disulphide bridges. Such a range of possible conformations (unlikely for α -helical regions lacking heptad repeats) may have implications for the antigenic variability of these proteins. Although the antigenic diversity may arise from amino acid replacements *per se*, it could also depend on structural changes, possibly gross, controlled by broken heptad repeats that can result from a small change of sequence.

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Mutant viral RNAs synthesized *in vitro* show altered aminoacylation and replicase template activities

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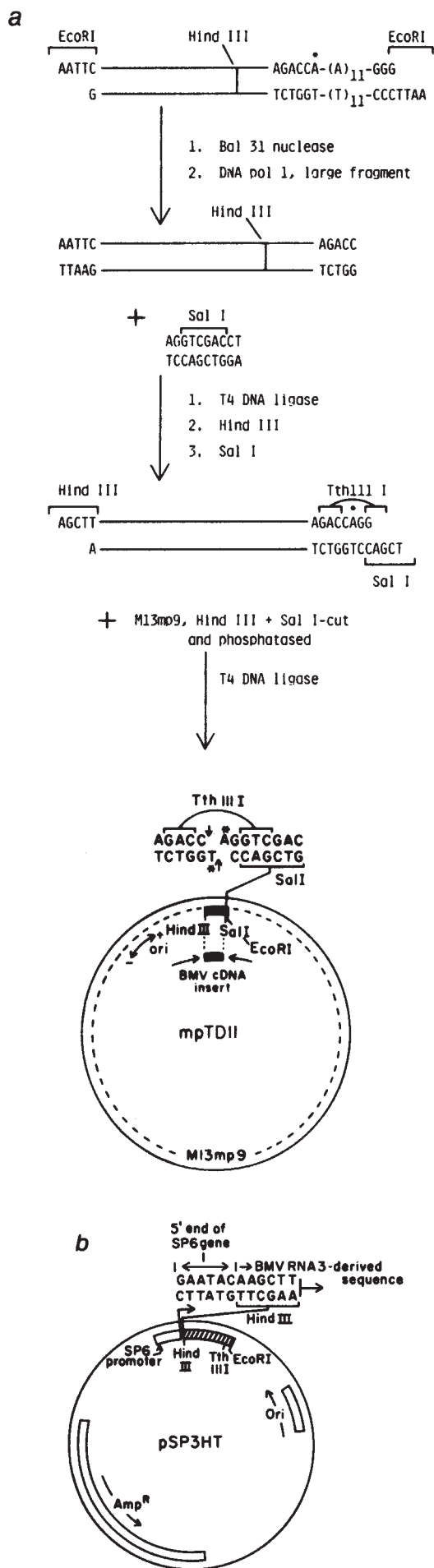
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A remarkable feature of the genomic RNAs of several plant viruses is the presence at the 3' end of a region that exhibits tRNA-like functions, including aminoacylation^{1–3}. The three genomic and single subgenomic RNAs of brome mosaic virus (BMV) accept tyrosine *in vitro*⁴ and *in vivo*⁵, the smallest 3' fragment that can be aminoacylated being about 135 nucleotides long⁶. The roles of the tRNA-like properties are incompletely understood, but an involvement in replication rather than translational functions is likely^{3,7,8}. We have recently shown (J.J.B. *et al.*, in preparation) that the features recognized by the BMV RNA-specific RNA-dependent RNA polymerase (replicase)^{9,10} for the use of BMV RNA for complementary strand synthesis also lie within the tRNA-like structure. To distinguish between the roles of BMV RNA as a substrate for tyrosyl-tRNA synthetase and BMV replicase, we have now produced BMV RNAs with mutations at two separate loci within the tRNA-like structure. This has been achieved by transcription *in vitro* from specifically mutagenized cDNA, an approach permitting the generation of targeted mutants without regard to their viability *in vivo*.

The synthesis of RNAs active in aminoacylation, a function known to be stringently dependent on a correct 3'-CCA terminus^{11,12}, required the extension of existing *in vitro* transcription techniques^{13,14}. Correct 3' termini were obtained by transcription from a template linearized at a position corresponding to the 3' end of the BMV cDNA sequence; a *Tth1111* restriction site was created at such a location within a 201-base pair (bp) BMV RNA3 cDNA clone containing the tRNA-like structure (Fig. 1a). After placing the BMV sequence under the control of an SP6 promoter^{13,15} (Fig. 1b), microgram quantities of BMV RNA transcript of the expected size (207 nucleotides) were prepared from *Tth1111*-linearized template DNA. The transcribed RNA was active in two assays characteristic of the tRNA-like structure of BMV RNA: aminoacylation and complementary strand synthesis by BMV replicase. The behaviour of BMV RNA transcripts in both assays was comparable to that of native virion RNA. These activities are discussed below in comparison with those of mutated RNAs.

Point mutations were introduced into the BMV cDNA segment of clone mpTD11 by oligonucleotide-directed mutagenesis¹⁶. Mixed oligonucleotide primers¹⁷, containing sequence ambiguities at two adjacent bases, were used to construct libraries expected to contain all possible single and double base mutants for each dinucleotide target. This procedure was applied to two loci in the BMV sequence, the 3' end and the –AUA–tyrosyl anticodon triplet. Figure 2a identifies the

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sequence substitutions of BMV RNAs produced from clones selected from mutant libraries.

Transcripts representing the wild-type and mutant BMV RNAs (207 nucleotides) migrated identically during electrophoresis (Fig. 2b). Termination of transcription did, however, show some heterogeneity, as indicated by the poorly resolved doublets. The 3' termini of all transcripts studied here were analysed by 3'-terminal labelling with 5'-³²P-pCp (ref. 18), followed by complete digestion with alkali or specific ribonucleases (data not shown). Although transcriptional termination on such linear templates gave rise to a limited range of products, it was clear that the heterogeneity existed beyond the required 3' end, that no significant premature termination was caused by the end of the DNA template, and that about one-third of transcript molecules consistently contained the expected 3' end. The run-off transcription system described here is thus capable of producing faithfully terminated RNA molecules.

Wild-type transcripts were aminoacylated with kinetics similar to those of virion RNA; about 40% of transcribed molecules were tyrosylated (Fig. 3a). This is consistent with an estimated one-third of transcribed molecules having a correct 3' terminus (see above). All five mutants in which the anticodon was changed to that of three different amino acids, or to the amber nonsense triplet, were as active in tyrosylation assays as were wild-type transcripts, and displayed indistinguishable kinetics (Fig. 3a). In contrast, all four mutations at the 3' terminus abolished aminoacylation. The low level of tyrosylation observed after longer incubations with mutant RNAs containing single base changes at the far 3' end was due to turnover of the 3' nucleotide¹², which generated some molecules with a correct -CCA end. tRNA nucleotidyl transferase and low levels of nuclease are known to be present in the partially purified synthetase preparations used.

The above sets of RNAs were assayed for their abilities to act as templates for BMV replicase (Fig. 3b). Wild-type transcripts showed similar activity to BMV RNA4, yielding full-length, double-stranded RNA. All of the point mutations studied

Fig. 1 Construction of a plasmid allowing the synthesis by transcription of RNA molecules comprising the tRNA-like region of BMV RNA3 and terminating in correct 3' ends. **a**, Scheme for the creation of a *Tth1111* site with the cleavage position coincident with the 3' end of the BMV RNA3 cDNA sequence. Clone mpTD11 contains the cDNA sequence corresponding to the 201 3' nucleotides of BMV RNA3. *Marks the 3' end of the BMV cDNA sequence. The cleavage positions for the *Tth1111* restriction endonuclease are indicated by arrows. **b**, Diagram of the transcriptional plasmid pSP3HT, obtained by subcloning the *HindIII* to *EcoRI* insert of mpTD11 (cross-hatched) into plasmid pSP64, a pUC12 (refs 25, 26) derivative containing an SP6 promoter fragment, and constructed in the laboratory of D. Melton. The 5' region of the DNA sequence transcribed by the SP6 RNA polymerase is shown; transcripts possess six bases at their 5' ends originating from the SP6 gene.

Methods: A fragment comprising the cDNA sequence corresponding to BMV RNA3 was released by *EcoRI* cleavage of 10 µg of a plasmid supplied by P. Ahlquist. To remove the poly(dA) tail added during cDNA cloning, the DNA mixture was treated with *Bal31* nuclease (0.6 units, BRL) at 16 °C in a reaction containing 20 mM Tris-HCl (pH 8.1), 0.6 M NaCl, 12 mM CaCl₂, 12 mM MgCl₂, 1 mM EDTA and 250 µg ml⁻¹ bovine serum albumin (BSA)²⁷. Portions of the reaction, taken at 7, 10 and 13 min, were pooled and the reaction was stopped in 20 mM EGTA, phenol extracted and ethanol precipitated. Treatment with *Escherichia coli* DNA polymerase I (large fragment, BRL)²⁸ was used to increase the proportion of fragments with blunt ends before linker addition. A decameric self-complementary oligodeoxynucleotide d(AGGTCGACCT), which contained an internal *SalI* site and was capable of creating a *Tth1111* site when ligated to the desired *Bal31*-deleted DNA, was synthesized by the phosphite triester procedure²⁹ using a DNA Synthesis Kit from New England Biolabs. The oligomer (0.6 µg) was phosphorylated to completion using T4 polynucleotide kinase (Pharmacia-P/L) in a reaction containing 200 µCi [γ-³²P]ATP (3,000 Ci mmol⁻¹; Amersham) chased with 500 µM unlabelled ATP³⁰. After ligation of the ³²P-labelled decameric linker to the *Bal31*-treated DNA, the DNA was digested with *HindIII* (BRL) and linkers were depolymerized by complete digestion with *SalI* (Pharmacia-P/L). Digestion products were electrophoresed through a 1.5% low melting point agarose gel, and a band (~200 bp) was extracted. The DNA was ligated to *HindIII* + *SalI*-digested, phosphatased M13mp9 vector²⁶ using T4 DNA ligase. Following transfection into *E. coli* JM101, clone mpTD11 (shown) was isolated by screening plaques for the presence of a *Tth1111* restriction site in replicative form DNA.

Fig. 2 Synthesis of BMV RNAs with mutations within the tRNA-like structure. **a**, Two-dimensional representation of the postulated conformation of the tRNA-like structure of BMV RNA3 (after Rietveld *et al.*³¹). Nucleotide positions are numbered from the 3' end, and the two pairs of adjacent nucleotides at which mutations have been introduced are boxed: the 3' terminus and the putative anticodon. Mutant clones were sequenced both before and after subcloning into the transcriptional plasmids, and the sequences of their transcripts are indicated for each locus; the amino acid corresponding to each anticodon triplet is also shown. The wild-type transcripts studied here varied from the RNA consensus sequence shown by the single base change G97 to U97 (indicated), which was also present in mutant transcripts. This substitution appeared neutral with respect to aminoacylation and BMV replicase template activity. Note that the split recognition sequence of *Tth*1111 GACN'NNGTC permits 3' mutations. **b**, Analysis of transcripts arising from mutated BMV cDNA clones on a 5% polyacrylamide-7 M urea sequencing gel. The migrations of DNA markers are indicated. Lanes 1-9, mutant transcripts 3'9, 3'64, 3'95, 3'98, A32, A48, A50, A60 and A74 respectively; wt is the wild-type transcript. Transcripts labelled with [α -³²P]UTP were synthesized from *Tth*1111-linearized transcriptional plasmids using SP6 RNA polymerase¹³.

Methods: Construction of mutant clones: Libraries of all possible double and single mutants at each locus were generated in a single mutagenizing experiment from single-stranded phage DNA (clone mpTD11), by an extension of published methods^{16,17}. 3'-Terminal mutations: A hexadecameric deoxyoligonucleotide mixture of sequence d(GTCGACCXYGTCTCTT) was synthesized as above. The positions designated X and Y were the sites directing the mutagenesis, where X = dT and Y = dG in the wild-type sequence. During synthesis, the condensation reaction at position Y contained a molar ratio of 3dC:3dA:3dT:dG of the protected nucleotide reagents. At position X, the molar ratios were 3dC:3dA:3dG:dT. In this synthesis system, molar ratios of condensation products are roughly proportional to the concentration of reagents in the reaction. The low proportion of the wild-type nucleotide was used to depress the ratio of recreated wild-type clones after mutagenesis. Conditions expected to allow priming of second strand synthesis by oligomers with double and single mismatches were established in pilot dideoxy sequencing reactions with the synthetic hexadecameric mixture as primer. The oligomer mixture was kinased and mixed with 1.5 μ g of single-stranded mpTD11 template (~15-fold molar excess of primer) in 9 μ l containing 20 mM Tris-HCl (pH 7.5), 10 mM MgCl₂, 50 mM NaCl and 1 μ M dithiothreitol. After boiling for 2 min, the mixture was annealed at 37 °C for 15 min, chilled on ice and brought to room temperature just before second strand synthesis. The reaction proceeded as recommended¹⁶. 100 phage clones were screened by temperature-shift hybridization, in which M13 phage was applied directly onto nitrocellulose (Schleicher & Schuell) and the [γ -³²P]-labelled synthetic oligomer mixture was used as probe^{16,17}. Hybridization was carried out at room temperature overnight, and was followed by 5-min washes at successively higher temperatures (37, 45, 55 and 60 °C), alternating with exposure of the dot blots to film. Suspected mutant phage were analysed by dideoxy sequencing³² using the M13 universal primer (17mer, New England Biolabs). Some phage selected on the basis of hybridization results did prove to be wild type. Because the blotted phage were not screened exhaustively, the proportion of mutants achieved was not determined. Anticodon mutations: The 21mer oligomer mixture used for mutagenesis at this locus was synthesized as above. The sequence was d(GACTTGCTWZGCAAGCCCATG), where W = dA and Z = dT in the wild-type sequence. The molar ratios at position W were 3dG:3dC:3dT:dA and at position Z were 3dG:3dC:3dA:dT. Mutagenesis was performed as above, except that the annealing temperature was 50 °C. During screening, washes were for 5 min at 50, 57, 67 and 72 °C. At least 8% of phage were mutants. Mutant clones were plaque purified, and the *Hind*III-*Eco*RI insert was subcloned into pSP64 as for the wild-type clone (see Fig. 1).

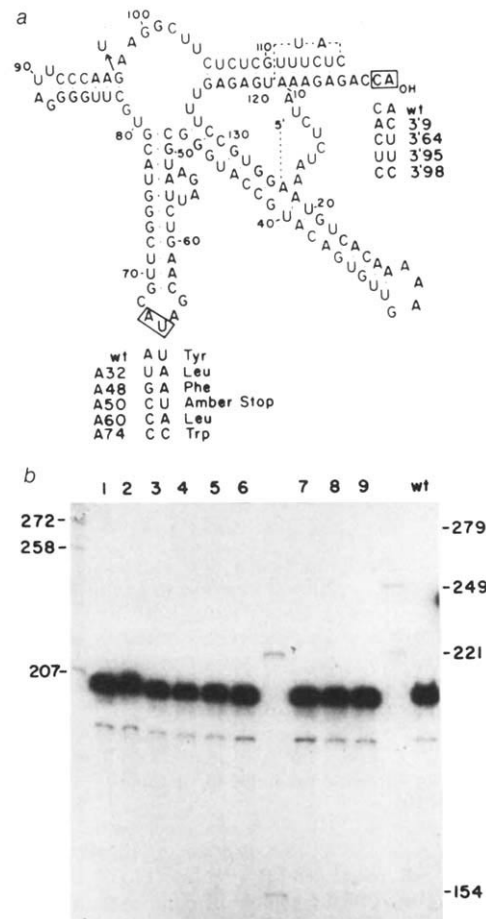


Table 1 Relative activities of mutant transcripts as templates for BMV replicase and in aminoacylation

Transcript	Relative replicase template activity	Relative aminoacylation activity
wt	1.00	1.00
3'9	0.05	0.00
3'64	0.34	0.02
3'95	0.05	0.01
3'98	0.32	0.01
A32	0.15	1.00
A48	0.15	0.97
A50	0.20	0.82
A60	0.18	0.94
A74	0.14	0.99

Replicase template activities were estimated by determining the ratio of ³²P c.p.m. incorporated into product by BMV replicase to ³H c.p.m., a measure of the amount of synthetic template (transcript) present. Replicase reaction products were phenol extracted and electrophoresed through 5% sequencing gels, conditions in which product and template co-migrate. Bands located by exposure to film were excised from the gel, and the RNA present was base hydrolysed using Soluene 100 (Packard), and analysed in a liquid scintillation spectrometer for ³²P and ³H c.p.m. Aminoacylation activities were derived from the amounts of ³H-tyrosine bound after 5-min incubations (Fig. 3a). This avoids the confusion with the end-group repair which occurs for transcripts 3'64 and 3'98 during longer incubations. Aminoacylation differences between anticodon mutants were not significant. wt, Wild type.

within the anticodon uniformly and markedly inhibited the template activity of the RNA. The template activity of 3' end mutants was governed by the location of the substitution. Transcripts with mutations only at the terminal position were active templates, although their activity was about one-third that of the wild-type transcript. In contrast, transcripts with substitutions at both the penultimate and terminal positions were very poor templates. The lowered activity of 3' mutant RNAs probably results from interference with the normal initiation of RNA synthesis.

The relative aminoacylation and replicase template activities of wild-type and mutant transcripts are compared in Table 1. Aminoacylation results were derived from charging levels after 5-min incubations, at which time 3' nucleotide turnover is undetectable (Fig. 3a). Replication activities were quantified by determining the ratio of product to template RNA in replicase reactions. Mutations at each locus had differential effects on the two activities. Several mutations within the anticodon triplet show that BMV replicase, and not tyrosyl-tRNA synthetase, interacts with this sequence, which exists in an exposed loop of the tRNA-like structure. The anticodon residues may thus be involved in the specific recognition of BMV RNA by the replicase.

The indifference of tyrosyl-tRNA synthetase to mutations in the anticodon of BMV RNA requires comment. Chemical modifications and mutations of the anticodons of tRNAs have not inhibited aminoacylation in all systems studied (compare

refs 19, 20). Further, although anticodons corresponding to the specifically bound amino acid are present in several plant viral RNAs^{2,3}, their occurrence is not universal; some viruses closely related to BMV, and whose RNAs bind tyrosine²¹, do not have analogous anticodons^{6,22,23}. This variation is compatible with the anticodon residues representing important determinants of replicase specificity. However, such observations leave open the question of the significance of the correspondence between bound amino acid and anticodon in RNAs from viruses such as BMV.

Four different mutations at the 3' terminus of BMV RNA abolished tyrosine-accepting activity. Thus, the -CCA terminus is as necessary for the aminoacylation of BMV RNAs as it is for tRNAs^{1,12}. In contrast, BMV replicase is not as stringent in its requirement for a correct 3' terminus, and mutants with substitutions in the terminal position retained 33% of replicase template activity. However, substitutions at both of the 3'-terminal positions resulted in 95% loss of activity. These results are consistent with initiation of (-)strand synthesis very close

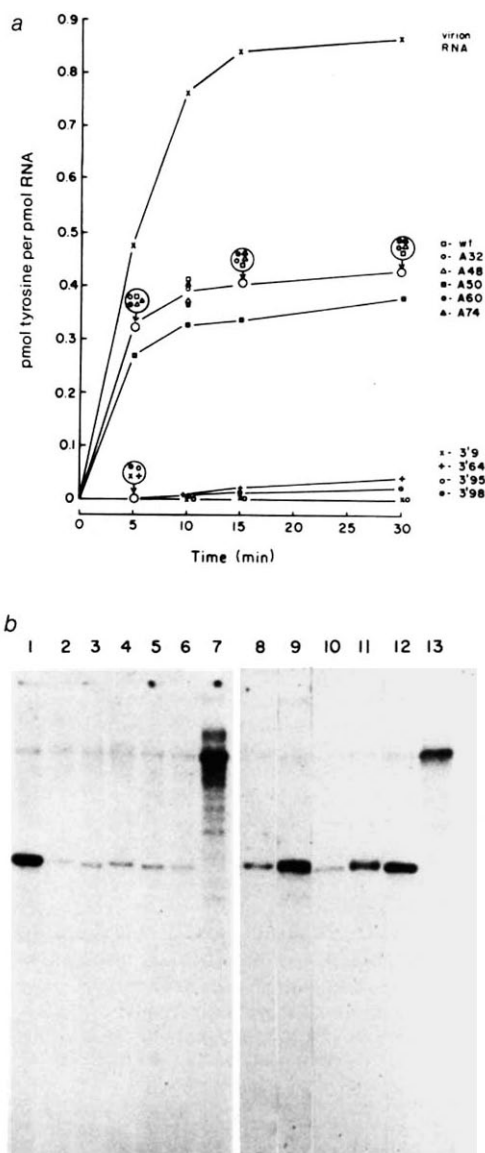
to the 3' end (J.J.B. *et al.*, in preparation), possibly at the penultimate C residue.

The approach described here for rapidly obtaining mutant RNA molecules with defined 3' termini is likely to be useful for studying the functions of various RNA molecules, including tRNAs, especially when the desired mutations cannot be obtained by traditional means or when the mutations are likely to be lethal. This system offers clear advantages over an RNA synthesizing system based on Q β replicase²⁴, which requires substantial Q β sequences at the 3' end of the RNA. Placement of cloned DNA sequences immediately adjacent to the SP6 promoter will permit the synthesis of RNAs with both authentic 5' and 3' ends.

We thank Dr M. Wickens for supplying SP6 RNA polymerase before its commercial availability, and for helpful discussions. Dr D. Melton supplied the transcriptional plasmid pSP64 and Dr P. Ahlquist supplied the BMV cDNA clone used. We thank Allen Miller for discussions, Dr L. A. Ball for help with the manuscript, Chris Harkin for technical assistance, and Professor

Fig. 3 Biological activity of wild-type and mutant BMV RNAs synthesized *in vitro*. **a**, Aminoacylation of transcripts. **b**, Activity of transcripts as templates for BMV replicase. Lanes 1 and 12, replicase reaction product with wild-type transcript as template; lanes 2–6, products with anticodon mutant transcripts as templates, A32, A48, A50, A60, A74 respectively; lanes 8–11, products with 3' mutant transcripts as templates, 3'9, 3'64, 3'95, 3'98 respectively. Lanes 7 and 13 are products obtained using 1.2 μ g BMV RNA4 as template; low levels of products synthesized from endogenous RNA4 are evident in the other lanes, and are useful internal controls.

Methods: **a**, BMV RNA was prepared as previously reported²¹. Transcripts were labelled to a known specific activity of ~60,000 d.p.m. per μ g with [α -³²P]UTP (Amersham), allowing calculation of the amount of RNA used in each aminoacylation reaction. Reactions (50 μ l) containing 3 μ g of *T7*1111-linearized DNA, 3.5 units of SP6 RNA polymerase and 500 μ M each nucleotide triphosphate typically yielded 3–5 μ g of transcribed RNA. RNA was phenol extracted and purified either using DEAE-Sephacel (Pharmacia) followed by ethanol precipitation, or by two successive ethanol precipitations in the presence of ammonium acetate. In both cases unincorporated nucleotides were completely removed. Because template DNA was shown not to interfere with the aminoacylation assay, it was not removed. Aminoacylation (tyrosylation) assays used a partially purified preparation of aminoacyl-tRNA synthetases from wheat germ³³. Reactions (50 μ l) contained 0.5–0.7 μ g of ³²P-labelled transcript RNA, 7.3 μ Ci ³H-tyrosine (54.6 Ci mmol⁻¹; NEN), 48 mM KCl, 5 mM MgCl₂, 0.64 mM ATP and 5 μ l of synthetase preparation and were incubated at 30 °C. Aliquots were taken at the times indicated, and incorporation of ³H-tyrosine was determined by precipitation of RNAs onto filter paper with trichloroacetic acid. Radioactivity was counted in a liquid scintillation spectrometer in conditions suitable for ³²P/³H double counting. The results shown are averages from two experiments for virion RNA, and of duplicate assays on at least two independently synthesized sets of transcripts. The altered sequences for mutant transcripts are identified in Fig. 2a. The results for transcript A50 were not significantly different from those of wild type or other anticodon mutants. **b**, Template activities of mutant and wild-type transcripts were assayed by incubation with BMV replicase, prepared from BMV-infected barley leaves and treated with micrococcal nuclease^{9,10}. Labelled complementary strand was synthesized in 25 μ l reactions containing 1 μ M [α -³²P]UTP (80 Ci mmol⁻¹; Amersham), 80 μ g ml⁻¹ actinomycin D, 20 μ l of BMV replicase preparation and template RNA. The reaction mixture was incubated at 30 °C for 30 min, extracted twice with phenol–chloroform, ether extracted and ethanol precipitated in the presence of ammonium acetate. A portion of the replicase reaction products was treated with ribonuclease⁹ before electrophoresis to confirm the presence of double-stranded products (not shown). Transcripts were prepared as above, but were labelled with [α -³H]UTP (NEN) to a specific activity of about 3×10^5 d.p.m. per μ g. Replicase reaction products with mutant and wild-type BMV transcripts and virion BMV RNA4 as templates were run on a 5% nondenaturing polyacrylamide gel and autoradiographed. Replicase reactions contained 0.9 μ g of transcript for 3'9 and 3'64 (lanes 8, 9), and 0.3 μ g for all others.



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Plasma protease inhibitors in mouse and man: divergence within the reactive centre regions

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The plasma protease inhibitors control a wide variety of physiological functions including blood coagulation, complement activation and aspects of the inflammatory response. The inhibitors function by forming a 1:1 complex with a specific protease within the reactive centre region of the inhibitor. Little is known about the evolutionary relationships of these inhibitors. We report here the sequences of cDNAs which represent the C-terminal halves of the two major murine plasma protease inhibitors. One of these, murine α_1 -antitrypsin, more appropriately called α_1 -proteinase inhibitor (α_1 -PI), has diverged from its human counterpart at a vital position in the reactive centre but this has not led to a physiologically significant change in function. Also, we have determined the partial sequence of a recently characterized protein termed contrapsin, which inhibits trypsin-like proteases^{1,2}. We show, surprisingly, that contrapsin is highly homologous to human α_1 -antichymotrypsin, an inhibitor of chymotrypsin-like proteases. The reactive centre regions of these two inhibitors have diverged considerably, which may account for the differences in specificity. We propose that the genes for contrapsin and human α_1 -antichymotrypsin are the descendents of a single gene that have evolved since rodent and primate divergence to encode proteins with different functions.

Previously, we have isolated cDNA clones corresponding to abundant liver-specific mRNA sequences³. Two of these clones, p1796 and p54, are complementary to mRNA encoding, respectively, the protease inhibitors α_1 -PI and contrapsin (our unpublished data and Jack Gaudie, personal communication). Figure 1 shows the DNA sequence analysis of the p1796 and p54 inserts. The two cDNA inserts are approximately 800 nucleotides long, representing the 3' half of the corresponding mRNA. Figure 1a compares the nucleotide and derived amino acid sequences of murine α_1 -PI with those published for the primate α_1 -PI (ref. 4). Over the region analysed, the mouse sequence is 70% homologous with the primate α_1 -PI at the nucleotide level, and 60% at the amino acid level. This degree of evolutionary divergence is common among many of the serum proteins studied^{5,6}.

Comparison of the sequence of mouse contrapsin (Fig. 1b) with that of murine α_1 -PI (Fig. 1a) reveals that the two inhibitors are related, sharing 59% homology at the nucleotide level and 44% at the amino acid level. This suggests that the two proteins diverged from a common ancestral gene probably some 200–300 Myr ago.

Contrapsin is a major component of the protease inhibitory activity of mouse plasma; however, this activity is not detected in human plasma¹. To search for a possible human counterpart, we screened a human liver cDNA library for complementary sequences⁷; approximately 0.1% of the clones screened gave a strong positive signal with the mouse contrapsin insert. One representative clone, pHL6.1, was selected and the region corresponding to the mouse cDNA insert sequenced. Figure 1b compares the mouse and human sequences, which are 70% homologous at the nucleotide level and 60% homologous at the amino acid level—the same degree of homology that murine α_1 -PI shares with its human counterpart. Analysis of the human sequence shows that it is identical to the published sequence of human α_1 -antichymotrypsin^{8,9} except for two amino acid differences. One of the amino acid differences places the termination codon 32 nucleotides upstream of the published triplet due to the addition of an A at nucleotide position 659. Therefore, murine contrapsin and human α_1 -antichymotrypsin are highly homologous.

Recently, a protease inhibitor 'superfamily' has been described which includes the human serine protease inhibitors α_1 -PI (ref. 10), antithrombin III (ref. 11) and α_1 -antichymotrypsin⁹ and two proteins having no reported inhibitor activities—chicken ovalbumin¹² and rat angiotensinogen¹³. We would now include mouse contrapsin in this 'superfamily'. We have aligned the sequences of all the members of this superfamily with our sequences and find that they have 15 amino acids in common. It is of interest that in all the members, except angiotensinogen, there is conservation of the two glutamic acid residues designated by arrows in Fig. 1a. Angiotensinogen contains only the first of the two residues. Two well characterized mutations^{14–17} that occur in human α_1 -PI, the S and Z polymorphisms which lead to low serum levels of the protein, result from substitutions at these sites. Conservation of these two amino acids suggests that these residues have an important role in the stabilization and secretion of this family of proteins.

Protease inhibitors are traditionally described according to their reactivity to proteases *in vitro* and the specificity of the inhibitor is determined by the reactive centre region of the protein. *In vitro* the protease may specifically cleave within the reactive centre between two amino acids at positions designated P₁ and P₁'. Studies involving substitution of different amino acids within the reactive centre suggest that the residue at the P₁ position is consistently the most important in determining the specificity of the inhibitor¹⁸; this was substantiated by a naturally occurring mutation in α_1 -PI called α -PI Pittsburgh, in which a single amino acid change at the P₁ position produced a protein having no α_1 -PI activity but possessing a several thousand-fold increase in antithrombin activity¹⁹ (Fig. 2).

The reactive centre regions of human α_1 -PI and α_1 -antichymotrypsin are located towards the C-termini of the proteins. Alignment of the mouse inhibitor sequences with the related

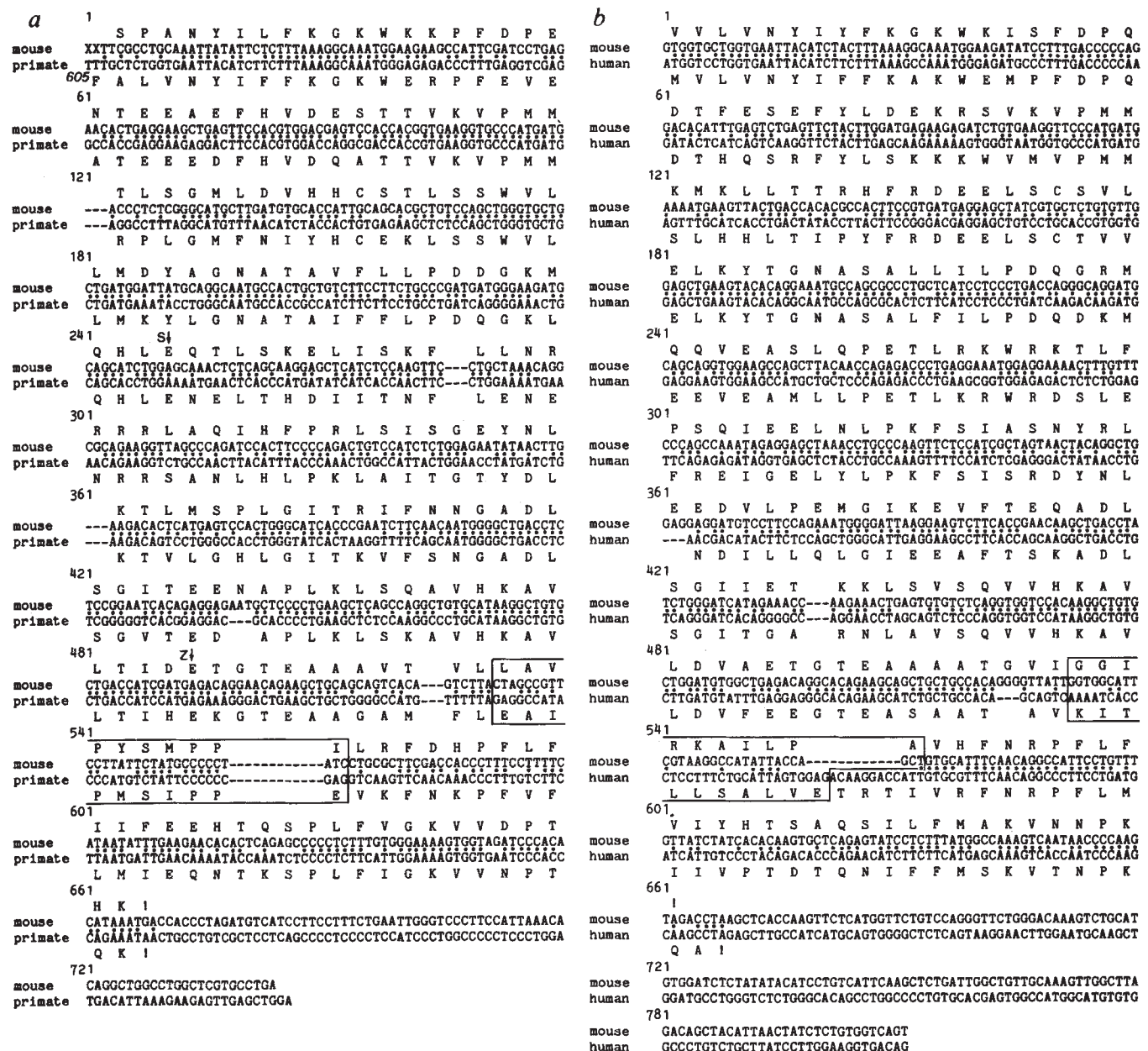


Fig. 1 The partial DNA sequences and derived amino acid sequences of mouse protease inhibitor genes compared with the corresponding primate or human protease inhibitor genes. All four sequences begin at the same relative nucleotide and were aligned, with gaps placed in regions of apparent additions or deletions of nucleotides. Amino acids are designated by the one-letter code. Dots indicate homologous nucleotides between the pairs of sequences. The 10 amino acids designated as the reactive centre regions of the sequences are boxed. **a**, Comparison of mouse and primate α_1 -PI nucleotide and amino acid sequences. The primate α_1 -PI sequence was taken from Kurachi *et al.*⁴ and starts at nucleotide 605 of the published sequence. The glutamic acid residues (E in the one-letter code) involved in either the S or the Z mutant forms of human α_1 -PI are indicated by arrows and the letter S or Z. **b**, Comparison of the mouse contrapsin and human α_1 -antichymotrypsin nucleotide and amino acid sequences. Mouse α_1 -PI and contrapsin and human α_1 -antichymotrypsin nucleotide sequences were derived by subcloning restriction fragments into the unique *Sma*I site of M13mp8 vector²⁶ and sequencing both strands by the chain termination method²⁷.

human sequences allowed us to predict the positions of the reactive centre regions in the mouse inhibitor (Fig. 1). The reactive centre regions of the human and mouse α_1 -PI differ by several amino acids (Fig. 2), most importantly, perhaps, at the P₁ position which in the human is a methionine and in the mouse a tyrosine. A major function of the human α_1 -PI is the inhibition of neutrophil-secreted elastase^{20,21}. Similarly, murine α_1 -PI is a potent inhibitor of mouse elastase ($K_i = 2.1 \times 10^{-11}$)², suggesting that the physiological specificity of these inhibitors is the same. However, the specificity of the murine α_1 -PI against some proteases *in vitro* differs from that of the human inhibitor. In this case, the difference at the P₁ position may affect the inhibitory properties of these two proteins *in vitro* but is probably of no physiological consequence.

In contrast, mouse contrapsin and human α_1 -antichymotrypsin differ greatly in their specificities; that is, contrapsin is active against trypsin-like proteases^{1,2} and α_1 -antichymotrypsin inhibits chymotrypsin-like proteases^{20,22,23}. Consistent with this, the reactive centre regions of these sequences differ substantially. Over the stretch of 10 amino acids that encompasses the reactive centre, one amino acid is shared (Fig. 2), with only 30% homology at the nucleotide level (Fig. 1b), whereas the nucleotide homology surrounding this region is strikingly high. At the P₁-P_{1'} position, the mouse inhibitor contains Lys-Ala residues in contrast to the human Leu-Ser residues. It has been predicted that an inhibitor with a lysine residue at the P₁ position would be more effective against trypsin-like proteases²⁴ than chymotrypsin-like proteases. The P_{1'} position

Protease inhibitor	P ₅	P ₄	P ₃	P ₂	P ₁	P ₁ '	P ₂ '	P ₃ '	P ₄ '	P ₅ '
mouse contrapsin	GLY	GLY	ILE	ARG	LYS /	ALA	ILE	LEU	PRO	ALA
human α ₁ -achy	LYS	ILE	THR	LEU	LEU /	SER	ALA	LEU	VAL	GLU
mouse α ₁ -PI	LEU	ALA	VAL	PRO	TYR /	SER	MET	PRO	PRO	ILE
human α ₁ -PI	GLU	ALA	ILE	PRO	MET /	SER	ILE	PRO	PRO	GLU
α ₁ -PI Pittsburgh					(ARG)					
human ATIII	VAL	ILE	ALA	GLY	ARG /	SER	LEU	ASN	PRO	ASN
BPTI	THR	GLY	PRO	CYS	LYS /	ALA	ARG	ILE	ILE	ARG

Fig. 2 Comparison of the reactive centre regions of several protease inhibitors. α_1 -PI Pittsburgh¹⁸, a mutant form of human α_1 -PI differing by one amino acid situated at the P₁ site and which has acquired antithrombin activity, is compared with human antithrombin III (ATIII)⁹⁻¹¹ which has the same amino acid at the P₁ site as α_1 -PI Pittsburgh. Bovine pancreatic trypsin inhibitor (BPTI)^{25,28} is an example of a protein with trypsin inhibitory activity having a P₁-P₁' site identical to that of mouse contrapsin. α_1 -achy, α_1 -antichymotrypsin.

is of interest here as the mouse protein is the only member of this family of inhibitors that does not have a serine at this position¹⁸; the functional significance of this is not known. However, a protein (bovine pancreatic trypsin inhibitor) (Fig. 2) from an unrelated family of protease inhibitors also has Lys-Ala at the P₁-P₁' position and, as predicted, exhibits antitrypsin activity^{24,25}. We propose, therefore, that mouse contrapsin is closely related to human α_1 -antichymotrypsin but has different inhibitory activity due to divergence within this short, functionally important stretch of amino acid residues.

In man, α_1 -PI and α_1 -antichymotrypsin are two of the most abundant serum protease inhibitors. In the mouse, the two most prevalent inhibitors are α_1 -PI and contrapsin. Attempts to demonstrate a contrapsin-like protein in man^{1,2} and an α_1 -antichymotrypsin-like inhibitor in rodents (H. Bauman, unpublished results) have failed. Thus, contrapsin and α_1 -antichymotrypsin are probably equivalent, and we propose that the genes for contrapsin and α_1 -antichymotrypsin are descendants of a single gene that have evolved, specifically within the reactive centre regions, to encode proteins with different activities. This has occurred since the time of rodent and primate divergence.

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Erythrocyte spectrin is comprised of many homologous triple helical segments

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Spectrin is an $\alpha\beta$ heterodimeric protein (molecular weight (M_r) = 460,000) which is a major component of the erythrocyte membrane skeleton¹⁻⁸. The membrane skeleton also includes actin (band 5) and is attached to the membrane via non-covalent associations with two linking proteins⁹⁻¹². Recently we have reported the amino acid sequence of a peptide of molecular weight 80,000 which comprises the NH₂-terminal one-third of the α subunit^{13,14}. This α -subunit peptide contains multiple homologous non-identical sequences with a periodicity of 106 amino acids and an approximate molecular weight of 12,000. It was also established that spectrin is not related to any other proteins whose sequence was known. We now report additional amino acid sequence of peptides representative of other domains of both spectrin subunits. The results suggest that most of the human erythrocyte spectrin molecule is comprised of homologous segments with a 106 amino acid (M_r 12,000) length per segment. Each homologous 106-amino acid segment may be folded into a triple helical structure with a short non-helical region connecting adjacent units.

Amino acid sequences from the α and β subunits which exhibit homologous structure are presented in Fig. 1. The sequences of the first nine repeats are derived from the α -1 and α -2 domains and provide nearly complete sequence information on the NH₂-terminal half of the α subunit.

The degrees of homology between each of the sequences in Fig. 1 were analysed using the computer programs ALIGN and RELATE with either an identity or mutational data matrix. In all pairwise comparisons of sequences, homology was statistically significant (s.d. > 5), and there was no evidence for any repetitive structure smaller than 106 amino acids.

When all homologous sequences are compared as a group, only a single amino acid, tryptophan at position 45, is completely invariant in all sequences. A number of other positions (indicated by arrows in Fig. 1a) are strongly conserved and might have critical structural roles. A single amino acid predominates in each of these positions with occasional conservative substitutions. Positions 1, 12, 15, 26, 35 and 46 contain hydrophobic residues, but unlike other positions where any hydrophobic residue is permitted, these six positions exhibit a strong preference for I, W, L, L, F and I respectively (single-letter amino acid code). Several other highly conserved positions involve charged residues which might be involved in critical salt bridges. Arginine is preferred at position 22, lysine occurs at position 71 and aspartate predominates at positions 38 and 41. Either glutamate or aspartate occurs at position 48 in most sequences. Histidine is strongly conserved at positions 72 and 101.

Figure 2 summarizes available sequence information. So far, the sequence of 1,435 residues or 35% of the total molecule has been determined. If most of the spectrin molecule is composed of 106-amino acid repeat units, approximately 38 repeat units would be present. Evidence of 22 homologous repeat units throughout both subunits has already been obtained, thus it is likely that most of the spectrin molecule is made up of a single type of repetitive structure. The positions of known sequences are schematically represented in Fig. 2b and c. Repeat units have been numbered starting at the NH₂-terminal ends of the α and β subunits. The two subunits are aligned antiparallel with respect to NH₂-terminal orientation when in the dimer form¹⁴⁻¹⁶. The NH₂-terminals of both subunits are blocked but these blocked termini are removed during mild trypsin treatment, and both the α -T80 peptide and the β -T74 peptide have free NH₂-termini. The COOH-terminal region of the β subunit beyond repeat 18 in Fig. 2 contains the four covalent

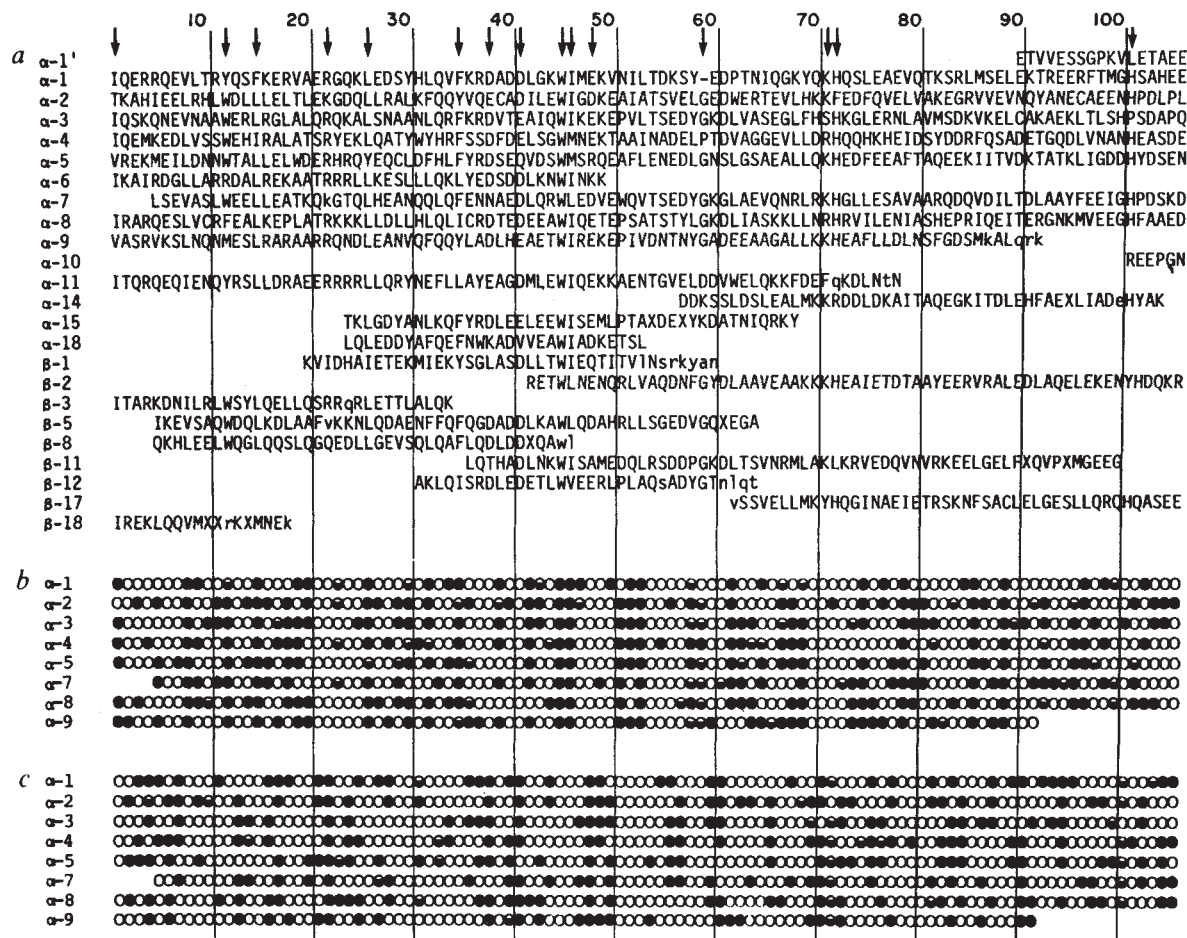


Fig. 1 The 106-amino acid repeat unit. *a*, Homologous sequences of the α and β subunits were aligned using the computer programs ALIGN and RELATE as previously described¹⁴ and are represented using the single-letter code. *b*, The locations of hydrophobic residues within the indicated α -subunit repeat units are represented. An extended hydrophobic series was used: ●—I, V, W, F, L, M, A, P; ○—Y, C, G, c. The locations of charged residues in the indicated eight repeat units are represented. ●—E, D, K, R; ○—H. The sequence (α -1' to α -6) of an α -subunit 80,000-dalton tryptic peptide previously named the α -1 domain¹³ and the sequence (α -7 to α -10) of a peptide of molecular weight 46,000 representing the α -II domain were determined using several overlapping groups of proteolytically cleaved peptides (D.W.S., G. Davis, V.T.M., manuscript in preparation). The remaining sequences with the exception of β -2, β -3 were obtained by extended automated Edman degradation of peptides which had previously been aligned by peptide mapping^{15,28}. The sequencing methodology used here has been described previously¹³. The peptides were produced by mild proteolytic treatment¹⁵, purified on two-dimensional gels and electroeluted²⁹, desalted by HPLC gel filtration in the presence of 0.2 M Tris-HCl, 8 M urea, 10 mM 2-mercaptoethanol pH 7.0 and dialysed extensively before sequence analysis. The amount of peptide used for sequencing ranged from 0.5 to 5 nmol per run. Because some of these sequences represent a single extended run, the COOH-terminal portions of these sequences should be regarded as tentative. The β -2, β -3 sequence is from an 11,000- M_r CNBr fragment near the NH₂-terminal of the β subunit which bound to calmodulin columns in the presence of 6 M urea (D. E. Sears, J. S. Morrow, D.W.S. and V.T.M., manuscript in preparation). Unidentified residues are represented as X and tentatively identified residues are represented by lower case letters. Sequences from each subunit are presented in the order in which they occur in the total sequence, starting from the NH₂-terminal end of the polypeptide chain. Optimal alignment of all the illustrated sequences within the 106-residue repeat unit required only a single one residue gap in α -1 at position 59. There is no simple way to define the start point or phase of the repeat unit without knowledge of the gene structure. We had initially assigned the start point based on the tentative hypothesis that the first 17 residues of the α -T80 peptide (α -1') were unrelated to the rest of the molecule¹⁴. We have since found that statistically significant correlations of homology cannot be made on 17-residue segments. It is unlikely that this phasing correlates precisely with either the gene structure or protein conformational units (see text and Fig. 3), but the current convention will be retained until more definitive data are available. Many sequences illustrated here are continuous with the next line and can be read in the same manner as text that is, sequences which extend to the right end of a repeat unit are contiguous with the left end of the consecutively numbered repeat. This continuity and the approximate placement of remaining sequence segments within the two spectrin subunits is diagrammatically represented in Fig. 2. Arrows indicate positions within the repeat unit which are especially well conserved in all sequences presented.

phosphorylation sites characterized by Harris and Lux¹⁶. This region is ~10,000 daltons and has not been sequenced. It may be unrelated to the general spectrin repeat unit, because its amino acid composition is quite different from the compositions of peptides having homologous structure (refs 14, 16 and data not given). One segment of the α -subunit (α -10) also appears to be unrelated to the basic 106-repeat unit.

The presence of repetitive sequences within the spectrin molecule is not novel although the large number of intramolecular repeats is somewhat unusual. A number of pro-

teins with internal repetitive structure have been identified, and current concepts of molecular evolution indicate that larger proteins have probably evolved from smaller precursors via gene fusions involving either unrelated genes or duplicated genes¹⁷⁻²⁰. In the latter case the resulting larger proteins have internal repeats which undergo independent mutation, evolving to non-identical but homologous sequences. Presumably spectrin has evolved in such a manner, and the large number of repeats suggests that spectrin evolution involved many gene duplication and fusion stages.

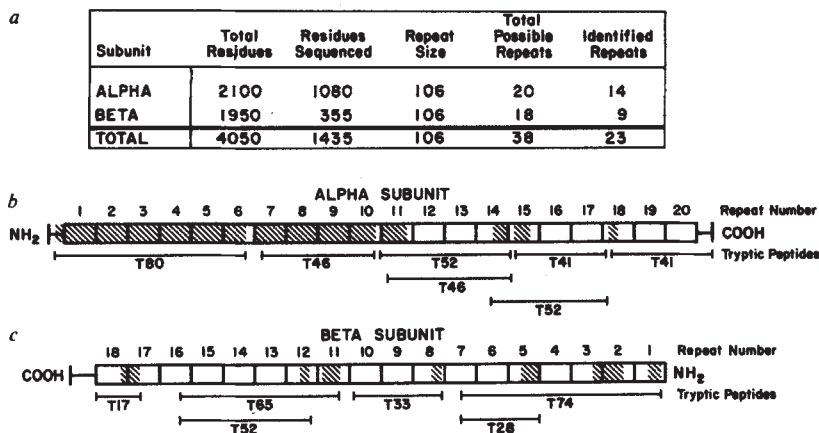


Fig. 2 Schematic representation of sequence positions within the spectrin subunits. **a**, Summary of sequence analysis. Several sequences are known in addition to the sequences represented in Fig. 1. These include 90 residues of the sequence from the α -10 segment which is not homologous to the rest of the molecule, partial sequence of the remainder of α -6, and several other short sequences. For simplicity each of the 106-amino acid segments will be referred to here as a repeat unit, and the apparently unique α -10 which may have been related to the remainder of the molecule at one time (see text) will be referred to as a non-homologous repeat unit. **b**, The α subunit (M_r 240,000) is represented as being made up of 20 repeat units (106 amino acids and 12,000 daltons per repeat) numbered starting at the amino-terminal end. Small regions near the NH_2 - and COOH -termini are believed to contain binding sites and these may be unique and unrelated to the repeat unit (ref. 14 and text). The positions of known sequences are represented by the cross-hatched areas. The tryptic peptides which were used for sequence analysis are represented and have been aligned based on previous peptide mapping analysis^{15,28}. The exact positions of sequences near the COOH -terminus of the α subunit are necessarily approximate and are based on three assumptions; (1) the α subunit is approximately 240,000 daltons and contains 20 repeats, (2) each repeat is 106 amino acids and approximately 12,000 daltons, and (3) several tryptic peptides overlap in repeat 14. This last assumption is the most tentative and is based on the consistently low yield of the T52 peptide encompassing repeats 14–17, the small number of spots on peptide maps involving repeat 14, and the fact that combined masses of the previously described domain peptides exceed the mass of the intact chain^{15,28}. There may also be additional overlaps between peptides represented here which have not yet been detected. Therefore the assignment of the indicated sequence segments to repeats 14, 15 and 18 are provisional but their relative order should be accurate. **c**, The β subunit (M_r 220,000) is comprised of 18 repeat units plus the phosphorylated region on the COOH -terminal end of the polypeptide chain. This subunit has been positioned antiparallel to the α subunit to illustrate their relative arrangement in the dimer, as the subunits associate side to side in an antiparallel orientation (see text). Repeats have been numbered starting at the NH_2 -terminal end. The positions of known sequences shown are represented by the cross-hatched areas and tryptic peptides which were used as sources of material for sequence analysis are also represented. The most ambiguous location of sequence segments in this subunit is the placement of the 11,000-dalton CNBr fragment in repeats 2 and 3. Based on mapping data, this sequence could either occupy the indicated position or be translocated 106 amino acids towards the COOH -terminal end and occupy corresponding portions of repeats 3 and 4. Remaining assignments of sequences seem to be unambiguous based on repeat size, peptide size and previous mapping data.

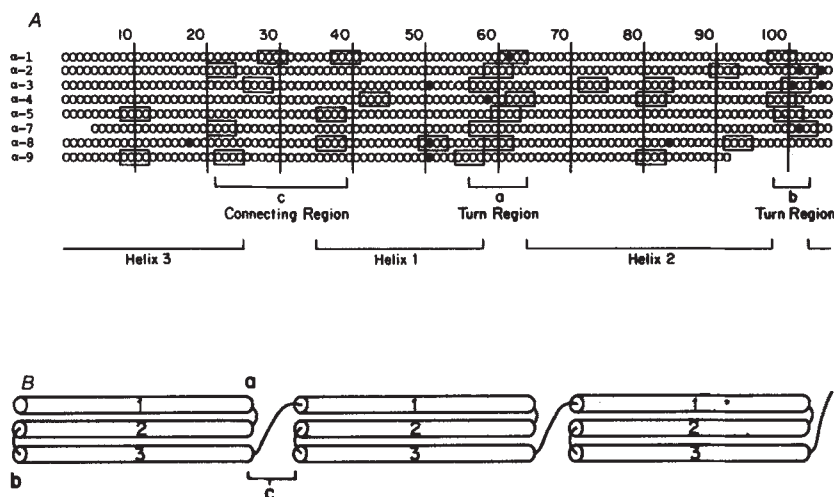


Fig. 3 Localization of reverse turns and connecting region within the 106-amino acid repeat unit. **A**, Diagrammatic representation of the eight repeat units of the α subunit corresponding to the sequences in Fig. 1. **B**, Three consecutive triple helical segments. The arrangement of helices, reverse turns (a and b), and the connecting region (c) between triple helical segments correspond to the similarly labelled regions of **A**. **Methods**: Reverse turns were calculated using P_{α} , P_{β} and P_T values, and positional frequencies for reverse turns as given by Chou and Fasman²². Average values of P_{α} , P_{β} and P_T were calculated over all possible 4-residue segments. Positional frequencies for reverse turns were calculated for 4-residue sequences with $P_{\alpha} < P_T > P_{\beta}$ and sequences with positional frequencies greater than 0.70×10^{-4} were scored as reverse turns if no other reverse turns with higher positional frequency values were predicted to occur within six residues. Two turn regions (a and b) were identified where most of the repeat units contained a predicted turn. A connecting region is also indicated where the helices probably break but are not in a turn configuration. Proline residues are indicated by shaded dots and the approximate locations of helical segments are given. It is quite likely that small amounts of other types of secondary structure also exist in the areas designated here as helices. One particularly interesting segment is between position 50 and 60 of the repeat unit. The entire segment between region c and turn a cannot be a single continuous helix in all repeats because three of the eight repeats in Fig. 4 have a proline at position 51. This would necessitate at least a kink in the helix at this point for these three repeats or it may signal a transition to another type of structure. Note that most of the repeats have a high β potential in this area¹⁴.

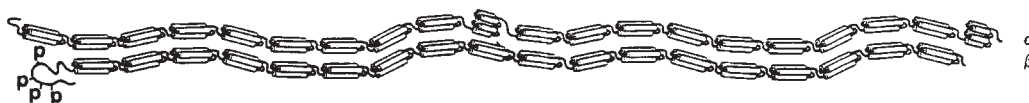


Fig. 4 A model of the spectrin dimer. The α and β subunits are composed of multiple triple helical segments connected by short non-helical regions. The subunits are antiparallel with the NH_2 -terminus of the α subunit on the left. Each triple helical segment contains ~ 106 amino acids, corresponding to the periodicity observed from sequence data (Fig. 1). The tenth segment in the α subunit is not homologous and probably has a more compact globular structure and is represented here by shorter helices simply to illustrate a more globular structure. The twentieth segment of the α subunit may have a common evolutionary origin to the tenth repeat (data not given) and hence is represented in a similar manner. The COOH-terminal region of the β subunit contains four phosphorylation sites and may also be unrelated to the triple helical repetitive structure.

The Chou and Fasman predictive method^{21,22} has previously been applied to an analysis of 595 residues of the α -1 domain¹⁴. This model can now be refined further, taking into account the existence of the 106-amino acid repeat throughout all domains of the molecule. Each of these repeats should have approximately the same conformation, as they were derived from a single ancestral gene, and it is well established that chain folding of related gene products is even more strongly conserved than amino acid sequence^{19,20}. This preservation of chain folding within spectrin repeat units is also suggested by the well defined zones occupied by either hydrophobic or charged residues (Fig. 1b, c). In most positions where hydrophobic residues occur, the zones are especially well defined. Two additional properties of spectrin provide essential information on the nature of spectrin secondary structure. The intact molecule and the isolated subunits are highly α -helical (60–80%), with little or no β structure as determined by circular dichroism measurements^{23,24}. In addition, the molecule is a long, thin, highly flexible rod $\sim 1,000$ Å in length^{25,26}, and this flexibility seems to be imparted by the presence of many hinge points throughout the length of the molecule. We also know that the NH_2 -terminus is near one physical end of each subunit and the COOH-terminal is near the opposite end^{14,15,27}.

The eight repeat units presented in Fig. 1b, c were analysed further to determine a protein conformational model (Fig. 3). Turn regions (a and b) have been assigned to the two areas in the repeat unit where turns were predicted in most of the repeats. A connecting region c, corresponding to at least part of the region centred at residue 30, is probably a short non-helical loop. These three regions (a, b, c) divide the 106-amino acid repeat into three parts corresponding to the helices of a triple helical segment. The size of the basic structural unit coincides with the basic genetic unit.

A model emphasizing important structural features of the spectrin molecule is given in Fig. 4. The α and β subunits are both made up of many homologous segments. Each segment, which contains 106 amino acids ($\sim 12,000$ daltons) is composed of a triple helical segment. The connecting region between adjacent triple helical segments is not helical and may provide the observed flexibility of the molecule. The two subunits are oriented in an antiparallel manner with the NH_2 -termini at opposite ends of the dimeric molecule.

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Friends and their foes

Gordon Conway

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By Charlie Pye-Smith and Chris Rose.

Penguin: 1984. Pp. 213. Pbk £3.95.

FOURTEEN years ago, when the British arm of Friends of the Earth (FoE) was founded, the world seemed to be heading for environmental disaster. The Friends were a tiny band, valiantly trying to stem the surging tide of pollution and bring wisdom to the management of dwindling resources. Fortunately, things have not turned out to be quite as bad as was forecast. Food production per capita has significantly increased in many Third World countries and birth rates are falling. We still have oil and coal in abundance. The end of the world is not going to come from ozone depletion and although increasing carbon dioxide and acid deposition may result in severe local disruption, the effects are unlikely to amount to a global catastrophe. There is still much to be worried about but, given the political will, solutions to most environmental problems seem feasible.

If the Earth is likely to scrape by, it might be presumed that the environmentalists would lose their mission and momentum. But FoE, although remaining a relatively small group of activists, is still a force to be reckoned with. In Britain, today's environmental crisis is more about the kind of world people want to live in, how desires can be satisfied, hazards and nuisances removed and fears allayed, than it is about imminent global catastrophe. It is a shift in emphasis to which the campaigners have adjusted and the politicians are beginning to respond. The British Government has announced its intention to reduce lead in petrol and to introduce pesticide legislation. David Owen and David Steel, leaders of the Social Democratic and Liberal parties, have recently signed a Greenpeace statement on radioactive discharges, and in William Waldegrave we now have a minister who gives the impression of being for, and not merely of, the environment. Industry too is accommodating. Pesticide legislation has been welcomed by the British Agrochemicals Association and the Confederation of British Industry is agreeable to much greater public access to information on polluting discharges.

Britain has been fortunate in the style and leadership of its environmental lobby. Targets are chosen with care, the homework is done and the propaganda contains no greater misrepresentation or oversimplification than is the norm for more respectable and traditional lobbies. Although fundamentally serious, the cam-

paigns have been witty and attractive, gaining broad public support.

In two of the chapters in *The Environmental Crisis*, Walter Patterson and Des Wilson recount the early and recent history of FoE. The battle roll extends from Rio Tinto Zinc, Schweppes returnable bottles, trade in endangered species, Save the Whale, recycled paper,

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REASONS

The Friends at work, defending the Somerset Levels against agricultural interests in 1983.

allotments, Windscale, the Wildlife and Countryside Act and lead-free petrol to today's campaigns against Sizewell and pesticides, and for greater freedom of information. Many of them have been successful and most people would agree we are better off as a result.

The book is subtitled *A Handbook...* but this is a misnomer. Facts and figures are included but are only sufficient to get the campaigner into an argument; there is not enough to sustain a case against well-informed opposition. There is little new in the book for anyone expert in the field. It is better read as a series of essays — on pollution, wildlife, land use, transport, energy, conservation and waste — by leading figures in FoE.

The most eloquent contribution is Richard Sandbrook's chapter on the environmental debate. Whereas much of the rest of the book is parochial in scope, Sandbrook addresses the global issues. He attacks those in the environmental movement who seek the salvation of the world through the creation of numerous small, self-contained islands. The reality, he argues, is ecological and economic interdependence and, in consequence, there are few, if any, simple solutions to our problems. If we are to achieve a more sus-

tainable and equitable world then there is no alternative to the hard grind of analysis and the energetic pursuit of solutions which, while not perfect, are at least a step in the right direction. One such step would be a sensible, long-term energy policy for Britain, and this proposal is well argued and supported in another chapter by Czech Conroy.

The most strident of the essays is by Chris Rose on "The Battle for the British Countryside". His arguments are elaborated in the second book reviewed here, *Crisis and Conservation*, co-written by Rose and Charlie Pye-Smith. This is a book about villains. Not surprisingly, chief among them are the farmers, forestry commissioners and water-authority members, together with their allies in the research institutes and universities. Less expectedly the Conservation Movement also comes under bitter attack. The Nature Conservancy Council, the Countryside Commissions and the Naturalists' Trusts are characterized as "dominated by people who are themselves farmers, foresters and landowners". Even FoE is criticized for being lured onto government committees.

The crime of this so-called "Old Guard" is that they believe farming and conservation can be reconciled. They seek consensus, cooperation and compromise while wildlife is being lost at an unprecedented rate. This seems to me unfair. There has been a long, and reasonably successful, tradition of voluntary agreements in British environmental policy. Such agreements, for example over the use of pesticides, can work providing the economic dice are not loaded against them. What has changed in the past few years has been the massive increase in the incentives for intensive farming consequent on Britain being subject to the Common Agricultural Policy (CAP). It is this which is making a policy of voluntary agreements ineffective and destroying the efforts of those who are working towards a compromise.

Pye-Smith and Rose provide a disturbing account of the destruction of British wildlife and habitats, but their book is weak on solutions. They believe planning controls are likely to be ineffective and they ignore the potential for changing the CAP. Their principal solution seems to be a land tax with exemptions for land of conservation or amenity value, yet there is no analysis of how this might work and it is not proposed with any confidence. The book ends with what amounts to a call for direct action: "Disorder, then, looks like being the order of the day". But disorder without policy or concrete solutions to put in place when the battle is won seems a mindless exercise. It will be a sad day if the environmental movement takes this road. □

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Foundations: fiction in fact

David E. H. Jones

Ascent to Orbit: The Technical Writings of Arthur C. Clarke.

By Arthur C. Clarke.

Wiley: 1984. Pp.226. £14.95, \$19.95.

WHAT makes a good science fiction writer? Apart from literary talent and uninhibited imagination, he needs one special gift. He must have a great knowledge and love of science, and yet not feel guilty if he is occasionally unfaithful to her. He must imagine, delight in and develop the consequences of things he knows to be scientifically incredible; yet to make them real he must embed them in a matrix of sound scientific understanding.

Few writers have these attributes, but Arthur C. Clarke is one of them. In dozens of stories, some of them major achievements of the genre, he has displayed his unruly imagination. Now in *Ascent to Orbit*, his collected technical works, he shows us his grasp of the rules.

The book brings together 24 technical articles spanning the years 1938–1981, on topics from electronics and astronomy to rocket theory and mathematical recreations. At its core is a cluster of about a dozen papers written between 1945 and 1950 on aspects of space technology. They form a sort of Old Testament in Dr Clarke's literary output: a foundation of rigorous theory in contrast to the later New Testament of wildly visionary science fiction. In them Dr Clarke, as a member of the British Interplanetary Society, is spokesman for a small band of enthusiasts preparing the way for a new technology. Escape velocities, the theory of rockets and their mass-ratio, transmitter powers necessary for interplanetary communication, theory of optimal orbital-transfer manoeuvres, are all here.

Among these papers is the brief, almost throw-away note which caused the whole book to be published. It is a privately-circulated two-page document dated May 1945 and entitled "The Space-station: Its Radio Applications". A few months later an expanded and now-famous version was published in *Wireless World*. In this paper Dr Clarke proposes the geostationary communications satellite, 20 years before the first one was put into orbit. This wonderful prevision of what is now the central technology of the "global electronic village" won him the 1982 Marconi Award for communication technology, which in turn inspired him to assemble the book. Dr Clarke modestly disclaims the title of "Father of Satellite Communications". He was, he insists, only its godfather.

It is tempting to scan this book for the seeds that were later to flower in his science fiction. There are some overt themes, of

course: space travel itself, ion-drive rockets, lunar colonies. But more subtly, the grain and detail of his fiction seem informed by the same respect for scientific and technical truth that is shown here. My favourite piece is not on space travel at all: it is "You're on the Glide Path — I Think", an account of his wartime experience as a development engineer with the first ground-controlled approach radar for aircraft landing. Dr Clarke describes the authentic feel of development work: the cams that revolved once during every approach unless they fell off their shafts, the calibration markers that vanished or were mis-identified, the aerial-rotation unit that chose the precise crucial moment to strip its gears. This is the sort of formative experience, I suspect, that gives his science fiction stories their credibility.

Arthur C. Clarke is, of course, an excellent writer. His style is effortlessly lucid and humorous, his papers always a pleasure to read. Some of them (for example his "Dynamics of Space Flight", first published in 1949) might well form a good introduction to their topics even today. His comments on them, interspersed throughout the book,

constitute an informal scientific autobiography which has a charm and interest of its own. One section describes his work as a physics-abstractor in 1949 and 1950: "I am proud of the fact that I appear as a distinct second-order differential coefficient on the curve 'Thousands of *Physics Abstracts* since 1900' in Derek J. de Solla Price's *Science Since Babylon*".

That was the last salaried job he held. His income from writing and broadcasting was rising, and he decided to make it his full-time career. His New Testament period was about to commence.

One might define a truly great science fiction writer as one who makes a dramatic technical prediction, incredible or absurdly visionary at the time, which within his lifetime actually comes true. By this criterion, Arthur C. Clarke and the communications satellite is up there with H. G. Wells and the atomic bomb. □

David E.H. Jones is a guest staff member of the Physical Chemistry Department, University of Newcastle upon Tyne, and a science consultant to industry and the media. He is author of The Inventions of Daedalus: A Compendium of Plausible Schemes, published by W.H. Freeman in 1982.

Mixed interferons

Robert M. Friedman

Interferons and Their Applications.

Edited by P.E. Came and W.A. Carter.

Springer-Verlag: 1984. Pp.575. DM 460, \$178.50.

IN THE past few years the cloning of the genes for human beta and gamma interferon, and for the subtypes of genes for alpha interferon, has both increased the supply of these interferons for clinical studies and led to the initiation of critical experiments on the control of the expression of this gene family. Many groups are now investigating the biological roles of the already well-characterized enzymes and active metabolites associated with the interferon system. Relatively recently, interferons have also been recognized as important immunomodulators and cell growth inhibitory substances.

Because of this wide range of biological activities, the publication of *Interferons and their Applications* is timely. The volume attempts "to catalogue the current understanding of interferons from different biomedical disciplines" in order "to assist the growing community of laboratory researchers and students who may come to share [the editors'] enthusiasm for long term, in-depth study of the interferon systems".

Sadly, however, the book does not attain these objectives, because of the manner in which it has been compiled; it is made up of 27 chapters of widely varied character and quality. Some chapters are excellent

reviews, some are for the most part reports of the author's own work, and others are philosophical discussions, but almost all of the contributions are too short to be really useful.

The difficulty of coordinating a large number of authors has also led to omissions, numerous repetitions and sometimes, even more unfortunately, to contradictions in the material presented. For instance, there is almost no discussion of the interesting effects of interferons on the replication of retroviruses. Extensive sections of Chapter 3 which deals with the evolution of interferon genes are repeated in Chapter 4 on comparative analysis of interferon structural genes. On p.73 of the latter chapter we read that: "It has clearly shown that the other human IFN- β genes are widely dispersed in the human genome", while the following chapter states: "In the contrast to the multigene family of Hu IFN- α , only a single Hu IFN- β gene has been found (p.85). Authors of other chapters specifically agree or disagree with each of these comments, thus compounding the confusion. Clearly a single discussion of the pros and cons of the thesis that there are multiple β interferon genes would have been more helpful.

Finally, the book has numerous typographical errors. All of the authors and the editors are culpable to varying degrees — but not many of them to the extent that they discuss work on "New Zeal and black (NZB) mice" (p.309). □

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Mineral physics from Japan

Raymond Jeanloz

Materials Science of the Earth's Interior.

Edited by Ichiro Sunagawa.

Terra Scientific, Tokyo/Reidel: 1984.

Pp. 653. Dfl. 340, \$120, £80.

How much good science can be purchased for 8×10^8 yen (approximately \$4 million at current rates)? Judging from this book, the answer is a lot.

Much of the leading research in mineral physics is being done in Japan, the work involving application of tools and principles from physics, chemistry, geology and materials science to the complex materials that make up our planet. *Materials Science of the Earth's Interior* summarizes research in mineral physics that was carried out during a three-year programme funded (with the amount mentioned) by the Japanese Ministry of Education. The programme was completed in 1981, and the book written in 1982, yet this volume is a remarkably up-to-date summary of work on the subject.

Although it emphasizes experiments over theoretical investigations, the book manages to cover a broad range of topics. For example, X-ray diffraction studies of silicate crystals and melts at temperatures up to $\sim 2,000^\circ\text{C}$ are complemented by accounts of molecular dynamics calculations on molten silicates. Descriptions of techniques available for growing high-quality crystals, including diamond and

other high-pressure phases, are followed by a number of studies on defects, rheology, diffusion and natural occurrences of the same minerals. High-pressure research is well represented, particularly in the areas in which the Japanese have excelled: the development and application of large-volume presses, *in situ* studies of phase transformations and determinations of complex phase equilibria. Also notable are individual contributions summarizing recent work on the theory of transition-metal compounds (Sugano and Ohnishi), the noble-gas geochemistry of natural diamonds (Ozima and co-workers), and the use of shock waves in high-pressure experiments (Syono and Goto). Overall, the articles bring together a great deal of material that was hitherto widely scattered or unavailable elsewhere in the literature.

The book will be especially interesting to the earth scientist who wants to know about advanced techniques that are now available and will become increasingly important in the study of geological materials. For the materials scientist or condensed-matter physicist, however, few clues are provided regarding the geological motivation for carrying out these studies; the connection between material properties and the evolution of planetary interiors is not explicitly examined. Still, the properties of complex inorganic systems such as minerals are of broad interest, and this book has allowed some of the leaders in mineral physics to describe frontier research in their field. □

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Beavering away

W.C. McGrew

Animal Architecture and Building Behaviour.

By Michael H. Hansell.

Longman: 1984. Pp. 324. £25, \$60.

TWENTY-THREE families of birds, from bulbuls to wrens, gather and use spider silk in constructing their nests. Beetles living in the Namib desert dig shallow trenches in the sand to trap dew for drinking water. Mole-rats burrow as a team, from the excavator in front, through the "conveyor belt" of intermediates, to the soil-expeller at the end of the queue. These are some of the splendid examples of animals building, as recounted by Michael Hansell. His book would be welcome if it were just an encyclopaedia of such habits, but it is much more.

A fifth of the text is taken up with a survey of animal architecture, across the minor builders from protozoa to chimpanzees, but focusing on the three main classes of major builders: spiders,

insects and birds. The rest is devoted to analysis of architecture as action. As an ethologist, the author considers the structure to be inseparable from the behaviour which creates it. He is a great lister: artefacts may serve four functions, namely, protection from the elements, defence against other organisms, aids to subsistence, and communication and display. (A beaver's dam may perform all four.) There are seven methods of construction — sculpting, piling-up, moulding, rolling and folding, sticking-together, weaving, and sewing — birds, apparently, having the widest range. With such classificatory schemes, the author makes neat sense of a diverse array of knowledge, so that unexpected generalizations emerge; for example, the same properties of mud are exploited by organisms from termites to house martins.

For the scientist, animal artefacts have many uses. As products of behaviour they can be used to infer the qualities of the minds that produced them. They can persist long after the acts were performed, thus providing "frozen" or "fossilized" behavioural data. Some artefacts are sufficient to describe taxa the character-

istics of which are otherwise completely unknown. Perhaps most impressive is that building has ramifications throughout the whole complexity of adaptation. Hansell argues convincingly that evolutionary changes in the choice of building materials may effect changes even in social structure; for example, in wasps the change from building with mud to building with vegetation allowed group sizes to increase and so for sociality to emerge.

The book is not without flaws. The author's special field of study is the house-building of caddis-fly larvae, and occasionally one feels that the book contains more than one ever wanted to know about these insects. By and large, vertebrates are under-represented compared with spiders and wasps — the use of hammer-stones by *Ammophila* wasps is mentioned four times, hammer-stone use by sea otters or chimpanzees not at all. The sleeping-nests of great apes rate only two sentences, despite an extensive literature which includes details of such things as the apparent use of dung as insulation against the cold by mountain gorillas!

A recurring problem is how wide to cast one's net, so to speak, in dealing with artefacts. The author is mostly interested in dwellings or other fixed structures, as his title suggests. Yet, at times, and seemingly arbitrarily, he inserts material on tool-use. The use of cafeteria-style trays by *Aphaenogaster* ants to carry off food is memorable, but why was this included when (for instance) probing with a cactus spine for insect prey by Galapagos finches was omitted? The author might better have left out tool-use altogether, as this has already been definitively dealt with in Benjamin Beck's *Animal Tool Behavior*, published by Garland in 1980 (for review see *Nature* 289, 616; 1982). In fact, the two books are largely complementary and show many resemblances in emphasis and viewpoint.

Overall, this is a book of thoughtful analysis, always enlivened with entrancing details of natural history. Everyone knows of the marvellous bower-birds of the Antipodes. But who was aware that one species changes the flowers in its bower daily, or that another spends hours trimming leaves above its arena so that sunlight can penetrate, enabling the bird to show off its plumage? □

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New in paperback

● *History of the British Flora: A Factual Basis for Phytogeography*, 2nd Edn, by Sir Harry Godwin. Publisher is Cambridge University Press, price is £18, \$36. For review see *Nature* 261, 527; 1976.

● *The Illustrated Natural History of Selborne*, an attractively-produced edition of Gilbert White's classic book, compiled by Ronald Davidson-Houston. Publisher is Papermac, price is £8.95.

If you go down to the woods today...

Paul Richards

The Primary Source: Tropical Forests and Our Future.

By Norman Myers.

W.W. Norton: 1984. Pp.399. \$17.95, £13.50.

TROPICAL forests are shrinking fast. Many believe that they may hardly survive into the next century, but books about them continue to multiply. Dr Myers's is only the latest in an outburst of publications which range from specialist volumes and authoritative texts, such as T.C. Whitmore's *Tropical Rain Forests of the Far East**, to semi-popular illustrated books and magazine articles.

The Primary Source is aimed mainly at non-specialists, but will also hold the interest of specialists. It is very readable and the author has the gift of explaining complex matters, such as the hydrological cycle of Amazonia, in simple, comprehensible language. Unlike most books on tropical forests, it is mainly concerned, not with the forests themselves, but with the

*The second edition of this book has just been published by Oxford University Press, price £40, \$67.50.

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impact of modern man on them — their destruction by commercial loggers, fuel-wood gatherers, cattle raisers and shifting cultivators — as well as with their actual (and potentially much greater) contribution to human welfare. Dr Myers is well qualified to write about this topic: he has spent many years in Africa and more recently made a comprehensive survey of the current state of "forest conversion" in the humid tropical countries on behalf of the U.S. National Research Council's Committee on Research Priorities in Tropical Biology.

Dr Myers interprets the term "tropical forests" very widely; indeed, when he writes about the economic possibilities of water hyacinths and capybaras one wonders whether his subject is not really the whole flora and fauna of the tropics. The opening chapters ("Bio-ecological Background") are the only ones dealing with the forest as such; they emphasize its richness in species and its ecological complexity. Some statements here are questionable, for example that on p.81 that the seeds of very few tropical trees are wind-dispersed; this is not true and overlooks the function of natural gaps in rain-forest regeneration. Overall the purpose of these early chapters might have been better served had more space been given to illustrations and less to trying to convey a written picture of what a rain forest is "really" like.

In the next section we are on firmer ground. Much of the chapter on commercial logging is fairly familiar, but Dr Myers gives figures on the insatiable demand of the United States, Japan and other advanced countries for tropical hardwoods which are both striking and disquieting, not least because it is evident that present rates of consumption cannot be maintained; already some tropical countries, such as Nigeria, are reserving what remains of their hardwood stocks for their own use. It is even more disturbing that less than one-twentieth of tropical forests are under management of any kind. A survey of logging companies showed that not one of them leaves enough trees to act as seed-parents for natural regeneration. The accounts of the cattle ranchers and fuel gatherers are also full of information but are no more reassuring.

Still more horrifying is the chapter on shifting cultivators (whom Dr Myers calls "forest farmers"). At least 200 million people depend for survival on the temporary and relatively unproductive farming of small plots won by felling and burning tropical forests. Their numbers are rapidly increasing and have been swollen by incursions of squatters and dispossessed people who lack the simple skills of the traditional forest farmers and are thus even more damaging to the environment.

The effects of human demands on tropical forests might be less tragic if their resources were used fully and rationally. But as the chapters on "Contributions to

our Welfare" show, this is far from true. In conventional tropical forestry, resources are classified into timber and "minor forest products". The latter now include such things as the steroid-yielding tubers of Mexican species of *Dioscorea* (yams) which are collected from wild plants in the forest and are an export worth millions of dollars. The future possibilities of discovering new drugs and insecticides in tropical forest plants may be enormous and others may provide templates or blue-prints from which such substances could be synthesized. Forest plants may also have uses as sources of protein and of "green gasoline" and other energy-yielding products. In order that the forest resources should be more completely, and less wastefully, used, Dr Myers suggests the establishment of "forest industrial complexes". Of course the products yielded by forests are not their only contribution to human welfare. Other chapters discuss their part in maintaining water supplies, and checking soil erosion and the silting of reservoirs, as well as their effects on the hydrological cycle, albedo and the carbon dioxide content of the atmosphere. The references on these and other topics are up to date and will be very useful for most readers, however expert.

Many readers will turn most eagerly to the final section of the book, "What We Can Do". The political, economic and biological problems are formidable, but Dr Myers thinks that there is much that can be done both by the citizens of tropical countries and by "outsiders". Basically he believes that the outlook would be brighter if tropical forests were valued at their true worth. Because they are undervalued, forestry departments have always been the Cinderellas of governments in tropical countries; agricultural departments are better supported, but often are more interested in cutting down than in conserving forests. More money is needed for forest protection and for research, and those who understand the importance of forests need to have an effective voice in governments.

Dr Myers sets out what all this implies in some detail, and even provides a "shopping list" for us outsiders which includes such activities as persuading giant corporations to do a better job for tropical forests. Gloomy though the prospects may seem, he remains optimistic, and points to success stories such as the more enlightened attitude to forestry in Indonesia and a change of policy in favour of forestry at the World Bank. But perhaps the most apposite message is the motto from Edmund Burke on the first page of the book: "Nobody makes a greater mistake than he who did nothing because he could do only a little". □

Paul Richards is Emeritus Professor of Botany in the University of Wales. He is author of *The Tropical Rain Forest*, reprinted by Cambridge University Press in 1979.

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Nucleic acids and related areas

The new chemicals and apparatus described this week are mainly for those working with recombinant DNA technology.

● Now available from Worthington, the Type II restriction endonuclease *CpfI* (from *Clostridium perfringens*) is an isoschizomer of *Sau3AI* and *MboI* which cleaves DNA leaving the 5' terminal extension GATC like *Sau3AI*, *BamHI*, *BglII* and several other enzymes. Like *Sau3AI*, *CpfI* cleaves DNA that is adenine methylated. The best conditions are 10 mM Tris-HCl, pH 7.5, 100 mM NaCl, 10 mM MgCl₂, 10 mM β -mercaptoethanol and 100 μ g ml⁻¹ bovine serum albumin at 37°C. It is supplied at 5–10 units μ l⁻¹ in package sizes of 200 units, 500 units or 1,000 units.

Circle No. 100 on Reader Service Card.

● Boehringer Mannheim has recently introduced three DNA-affinity ligands which have been developed specially for the sequence specific electrophoretic separation of DNA fragments. By the use of two-dimensional gel systems, DNA fragments of differing base composition may be separated according to their retarded mobilities in the presence of a particular ligand. After separation the ligand can be removed, yielding the unmodified DNA. Also available from Boehringer Mannheim is a top quality Ribonuclease H. The enzyme is particularly suited to cDNA preparation. The absence of endonucleases and nicking activity is routinely checked by incubation with λ DNA and pBR322 DNA followed by gel electrophoresis. Absence of RNaseIII and RNase is checked by incubation of the enzyme with tritiated poly (U) poly (A) and MS 2RNA.

Circle No. 101 on Reader Service Card.

● Bio-Rad's new Mini-Sub submarine electrophoresis cell speeds the separation of DNA restriction fragments and other nucleic acids. Using very small sample loads, high resolution is obtained in runs of only 10 cm, taking only 30 min to 2 h. Effective separations of *EcoRI* or *HindIII* digest of λ phage DNA have been performed at 175V in as little as 20 min. Using sample loads as small as 50 ng, as little as 1–5 ng of DNA can be detected in agarose gels with ethidium bromide, while as little as 500 pg of DNA can be detected in polyacrylamide gels using the Bio-Rad Silver Stain. The Mini-Sub cell's increased speed and sensitivity simplifies many procedures. For example, clones can be screened rapidly for proper recombinant inserts, DNA enzymes can be assessed for activity, and DNA ligations, and other DNA modifying reactions can be monitored quickly.

Circle No. 102 on Reader Service Card.

● Terminal transferase and AMV reverse transcriptase are now available from Boehringer Mannheim. Both enzymes are extensively tested for the absence of endonucleases, exonucleases and nicking activities. Boehringer also markets SP-6 polymerase and SP-62 PL plasmid DNA. The SP-6 polymerase system generates single RNA species of known genetic origin in large quantities. These RNAs may be used as highly specific probes in blot and *in situ* hybridizations and as substrates in RNA transcription studies and S₁ mapping experiments. The enzyme is supplied free of contaminating endonucleases, exonucleases, nicking activities and ribonucleases. Restriction endonuclease *Asp718* (G/GTACC) is also available from Boehringer Mannheim. *Asp718* is an isoschizomer to *KpnI*, but produces fragments with 5'-cohesive ends as opposed to the 3'-cohesive ends from *KpnI*.

Circle No. 103 on Reader Service Card.

● A new 24-page catalogue includes detailed descriptions and prices for Becton Dickinson's complete line of monoclonal reagents, kits and panels for enumerating and identifying human and mouse leukocytes and immunoglobulins. Second-step reagents and monoclonal controls are also listed.

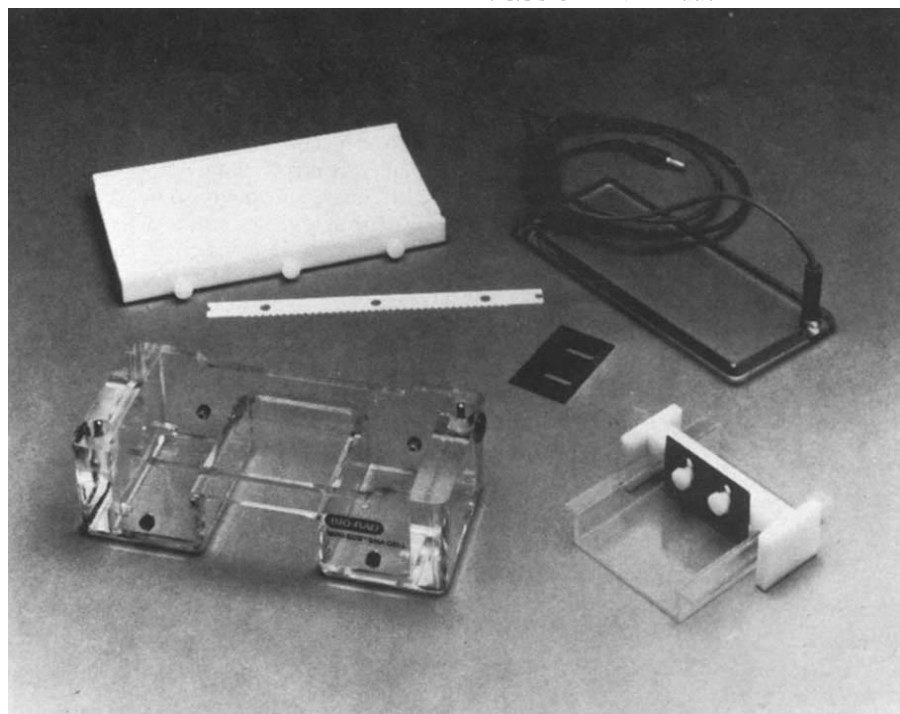
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● A comprehensive range of restriction endonucleases, DNA vectors and modifying enzymes is marketed by Genetic Research Instrumentation of Bishop's Stortford, UK. The company also offers a complete range of instruments and biochemicals for all aspects of molecular biology — for plant cells as well as animals and microbes. The new range of enzymes is added to the existing range of plant protoplast enzymes.

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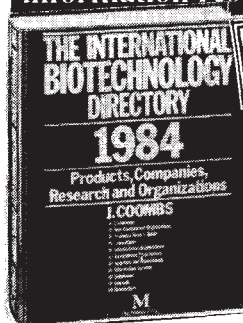
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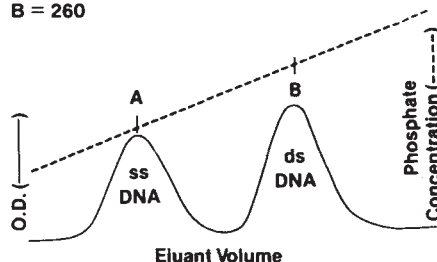
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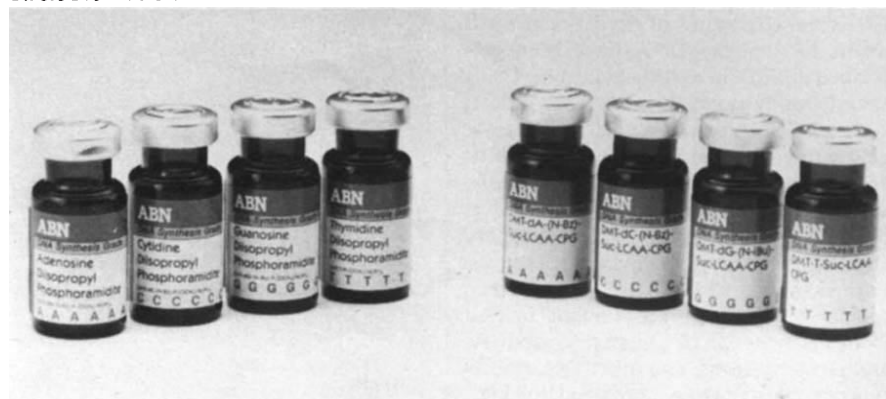
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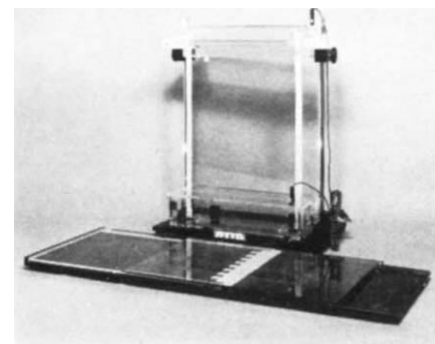
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• American Bionuclear's DNA Synthesis Grade diisopropyl phosphoramidites is suitable for preparing synthetic oligonucleotides using either manual or automated procedures. Each preparation is more than 98% pure based on ³¹P NMR and TLC analysis. Functional tests conducted on each lot guarantee greater than 95% coupling efficiency. A detailed quality control assay report is included with each shipment. The diisopropyl phosphoramidites are distributed in septum vials designed to fit commercially available automated DNA synthesizers.

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• Schleicher & Schuell's 'Quick Blot Kit' is a highly selective, quantitative and rapid method for the purification and immobilization of multiple samples of mRNA onto nitrocellulose. mRNA isolated from small volumes of whole cells, bacteria and virus can be hybridized, translated, reverse-transcribed or released and manipulated. The mRNA is tightly bound and always remains biologically active as no baking of the membrane is required. The system takes advantage of the high selectivity of nitrocellulose for mRNA which has been dissolved in concentrated NaI. Under these conditions and at low temperatures, adsorption of mRNA is very high whilst rRNA, tRNA, tRNA, native and denatured DNA, as well as proteins do not co-immobilize.

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PRODUCT REVIEW

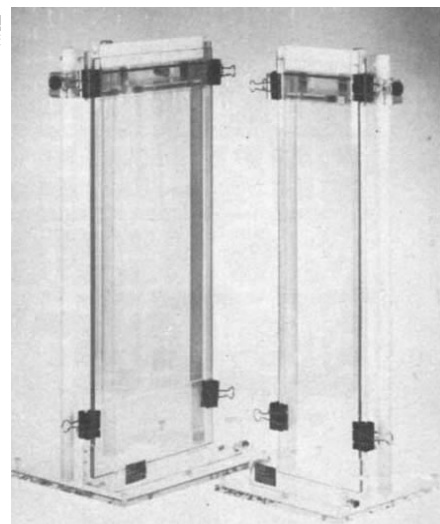
● D-Gel, a new product from Tekmar, will rapidly and efficiently extract nucleic acids from gels. This electrophoretic transfer system will directly transfer nucleic acids to DEAE-cellulose, or DEAE anion-exchange resin. DNA can then be recovered rapidly and intact in a quantitative yield, free from contaminating gel impurities, for assured biological activity in subsequent enzymatic reactions. The system ensures a high recovery efficiency — nanogram to submilligram quantities — with no danger of cross contamination. Utilizing isolated and removable transfer chambers, the D-Gel system is compatible with standard laboratory power supplies. *Circle No. 111 on Reader Service Card.*

● New additions to Cappel's range of affinity gels are anti-mouse IgG(H&L) coupled to Sepharose 4B and anti-rat IgG(H&L) coupled to Sepharose 4B. The antibodies are affinity purified and the mouse affinity gel will bind mouse IgG1, IgG2a, IgG2b, IgG3, IgM and IgA; the rat IgG affinity gel will bind rat IgG1, IgG2a, IgG2b, IgG2c and IgM. Affinity gels are useful for purification of mouse or rat immunoglobulin as well as immunoprecipitation of any IgG subclass mouse or rat monoclonal antibodies. These gels may also be used in columns for the same purposes. Each millilitre of gel has bound to it 2.0 mg of active affinity purified antibody resulting in a binding capacity of 1.0 to 1.75 mg ml⁻¹ of IgG. *Circle No. 112 on Reader Service Card.*

● The new DNA sequencing system from American Bionuclear is available in two versions: SQU-1000 (20 cm width) and SQU-2000 (33 cm width). Both are fully adjustable to provide the flexibility of running sequencing gels from 20 cm to 1 m in length. Both models feature adjustable upper buffer reservoirs, a 'no leak' gasket system of high compression silicone tubing that guarantees a tight seal and leak-free direct buffer-gel contact. Ports on both the upper and lower reservoirs permit buffer re-circulation, and a wide range of accessories is available for both models. *Circle No. 113 on Reader Service Card.*

● The LKB 2301 Macrodrive 1 power supply is intended for low-voltage electrophoresis applications. Using a few simple switches and clear, easy-to-read LED displays, the required constant voltage (0–1,000 V), current (0–400 mA) and integral digital timer (0–60 hours) can be quickly set. The Macrodrive 1 is well suited for routine PAGE, SDS, disc and gradient gel electrophoresis, as well as immuno-electrophoresis. *Circle No. 114 on Reader Service Card.*

● Purpose-built for the new blotting techniques the Desatronic 200/1000 (0–200 V/O–1,000 mA) power supply is available from Desaga GmbH of Heidelberg. Also new is the Desatronic 6000/100 for isoelectric focusing. Voltage, current and wattage are regulated automatically; all parameters are digitally displayed. *Circle No. 115 on Reader Service Card.*

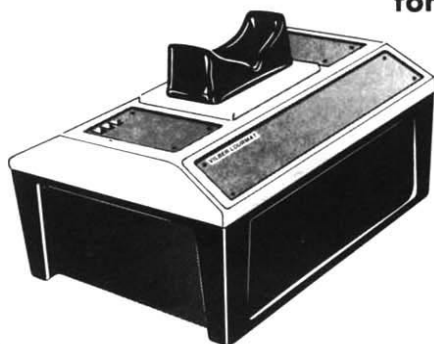


Top: American Bionuclear's DNA sequencing apparatus comes in two sizes. Bottom: Tekmar's D-Gel for extracting nucleic acids from gels.

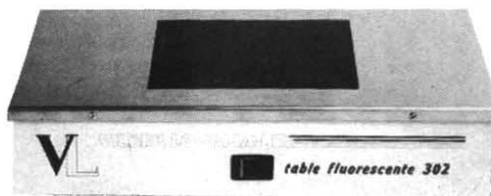
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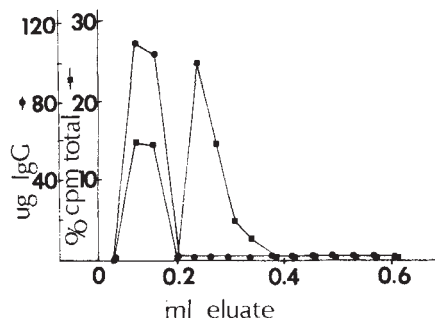


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Separation of Protag-125-iodinated human IgG from unbound ^{125}I on a Baker-10 SPE dextran gel column.

● A new approach to protein and peptide ^{125}I -iodination is now available with Protag-125 from J.T. Baker Chemicals. Protag-125 is a solid-phase iodination reagent that is supplied in an easy-to-handle powder form. Compared to existing techniques, Protag-125 is claimed to offer greater speed, higher efficiency and lower exposure to ^{125}I .

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● High-purity custom oligonucleotides are now available from Systec Inc. of Minneapolis. Synthesized at over 99% per step coupling efficiency on automated synthesis equipment, the price per condensation is \$30.00. There is no basic setup charge and no extra charge for degeneracies (mixed probes). Multiple sequence discounts are also available. Circle No. 117 on Reader Service Card.

● Two strains of *Escherichia coli* are now available from Bethesda Research Laboratories (BRL) as "Frozen Competent Cells", HB101 (RecA⁻) and RRI (RecA⁺). Prepared by a method based on the procedure of D. Hanahan (*J. molec. Biol.* **166**, 557-580; 1983), Frozen Competent Cells are ideal for cDNA cloning and other procedures requiring high-efficiency transformations. The cells need no preparation prior to transformation. They are simply thawed on ice before use. Unused cells can be refrozen up to five times with no significant loss of efficiency. BRL Frozen Competent Cells have provided greater than 1×10^8 transformants per microgram of supercoiled monomer pBR322, as compared with only 1×10^6 transformants of the same vector that can be achieved using competent cells prepared by the commonly-used calcium chloride treatment. Although efficiencies vary for ligations and cDNA cloning procedures, BRL claims that efficiencies are routinely greater with Frozen Competent Cells than with calcium chloride-treated cells. The cells are thoroughly performance-tested with supercoiled monomer pBR322.

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● Two new vectors, originally developed by Dr Guido Volkaert, have been added to the Amersham International range. The vectors provide flexibility and time-saving for Maxam and Gilbert sequencing, enabling plasmid "mini-preps" to be used in chemical degradation sequencing reactions after a single restriction enzyme digest and a short "filling-in" reaction. Any insert cloned into these plasmids is bounded by two *Tth*111 I restriction sites (GACNNNGTC). Digestion with *Tth*111 I generates four different 5' protruding ends. A simple Klenow reaction in the presence of only one labelled dNTP (for example dCTP) therefore specifically labels only one strand of the insert; the other strand can be labelled by including an alternative dNTP (for example dTTP) enabling the insert to be easily sequenced from both ends. Amersham's two plasmids ready for immediate cloning are: pGV401 *Pst* I-cut, oligo(dG) tailed (code RPN.1251) and pGV403 *Sma*I digested and dephosphorylated (code RPN.1252).

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● Video Blink, from Artek Systems Corporation, is a convenient and efficient method for identifying qualitative differences between two-dimensional electrophoretograms. Magnification of images facilitates the alignment of images and detection of faint spots. The loss or gain of a protein causes a spot to blink.

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Awards

Professor K.D. Cole of Latrobe University, Melbourne, Australia, has been awarded the 1984 **Appleton Prize** for Ionospheric Physics by the Royal Society UK, in recognition for his contributions to understanding the fundamental processes taking place in the magnetosphere.

The Royal Society has also elected the following as new Foreign Members:

Professor P-G. De Gennes, Professor at the Collège de France, Paris, in recognition of his many major contributions to theoretical physics — in the fields of magnetism, superconductivity, liquid crystals, polymers and mixed fluid flows; **Professor R. Hoffmann**, John A. Newman Professor of Physical Science at Cornell University, Ithaca, New York, USA, in recognition of his contributions to theoretical chemistry, particularly as originator of the extended Huckel theory; **Professor M. Meselson**, Cabot Professor of Natural Science, Harvard University, Cambridge, Massachusetts, USA, in recognition of his first demonstration of semiconservative DNA replication and for his studies on the mechanism of genetic recombination; **Professor G.E. Palade**, Professor of Cell Biology at Yale University, New Haven, Connecticut, USA, in recognition of his investigations of the ultrastructure of biological cells, and notably the description and the delineation of the functions of the endoplasmic reticulum; **Professor C. Rubbia**, Professor of Physics, Harvard University, Cambridge, Massachusetts, USA, and Senior Research Officer, CERN, Geneva, Switzerland, in recognition of his work as an initiator of important experiments in new areas of high energy physics, and **Professor H.H. Ussing**, Professor of Biochemistry at the University of Copenhagen, Denmark, in recognition of his work on the active and passive transport of ions across cell membranes and on the functioning of secretory epithelia.

Emeritus Professor Sir Alan Harris, FEng of Imperial College London, has been awarded the Gold Medal of the Institution of Structural Engineers.

Professor J.T. Stuart, FRS, Head of the Department of Mathematics at Imperial College, has been awarded the Whitehead Prize of the London Mathematical Society.

Professor Michael Sela, President of the Weizmann Institute of Science, **Professor Louis Chedid** of the Pasteur Institute, Paris and **Professor Edgar Lederer** of the French Academy of Science, have been jointly awarded the Prix de l'Institut de la Vie by the Fondation Electricité in France for their elucidation and perfection of the production of synthetic vaccines and the adjuvants of immunity.

Mr Erik Quistgaard, Director General of the European Space Agency, has been presented with the NASA Distinguished Public Service Medal.

Dr Richard Beasley of Wellington University, New Zealand, has been awarded the first two-year fellowship by the Wellcome Trust in association with the Medical Research Council of New Zealand, to study factors underlying asthma — at the Department of Medicine, Southampton University.

Appointments

Sir Alec Merrison and **Professor L.A. Thomas** have been elected President and Vice-president, respectively, of the Institute of Physics, London.

Dr John Midwinter, Head of the Optical Communications Technology Division of British Telecom, has been elected to the British Telecom Chair of Optoelectrics at the Department of Electronic and Electrical Engineering, University College London.

Professor Brian May, Head of Silsoe College, Bedfordshire, UK, has been elected President of the Institution of Agricultural Engineers.

Professor Guillermo Soberón Acevedo, Secretary of Health and Welfare, Mexico, has been elected President of the 37th Annual Session of the World Health Assembly (WHO), Geneva, Switzerland.

Professor Edwin C. Cadman, MD, Professor of Medicine at the University College of San Francisco, has been elected to the presidency of the American Federation of Clinical Research.

Dr Gordon H. Sato, Director of the W. Alton Jones Cell Science Center, has been elected a Member of the National Academy of Sciences, USA.

James F. McDonald has been elected President and chief operating officer of Gould Electronics, Illinois, USA, while **David Simpson** has been elected vice-chairman of the board.

Professor Roderick N.M. McSween has been appointed to the Chair of Pathology at the University of Glasgow; also at Glasgow University, **Professor Kenneth C. Calman** has been appointed to the Chair of Postgraduate Medical Education and the Deanship of Postgraduate Medicine, and **Dr Miles D. Hounsley** has been appointed to the Gardiner Chair of Biochemistry.

Professor Gilmour Toner has been appointed to the Musgrave Chair of Pathology, and **Dr Ronald Henry Perrott** to the Chair of Software Engineering at the Queen's University of Belfast, Northern Ireland.

Professor Bryan Carsberg of the London School of Economics has been appointed as Director General of Telecommunications, UK.

Dr John Rose, a Director of Dainichi Sykes Robotics Ltd, has been appointed to the United Kingdom's first University chair in automated manufacturing technology at the University of Salford.

Arnold Allen, CBE, a Chief Executive at the UK Atomic Energy Authority, has been appointed its full-time Chairman.

Michael Bown, formerly Group President in charge of International Operations at Peabody International Corporation, has been appointed as the first Director of CEED — the UK Centre for Economic and Environmental Development.

Dr George Russell, Senior Lecturer in the Department of Electrical and Electronic Engineering, Heriot-Watt University, has been appointed to a new Professorship within that Department.

Dr Bernard Wegener has been appointed Managing Director of Merrell Dow Pharmaceuticals GmbH, Ruesselsheim, West Germany.

Dr Bernard Hyams of the UK has been appointed by the Council of CERN — The European Laboratory for Particle Physics near Geneva, Switzerland — as the new head of the Experimental Physics Division.

Erich Bloch, currently Vice President for Technical Personnel Development at IBM, has been appointed Director of the National Science Foundation (NSF).

Dr Moustafa T. Chahine has been appointed to the position of Chief Scientist at the Jet Propulsion Laboratory (JPL), NASA, California; he is currently Manager of the Earth and Space Sciences Division at JPL.

Paul F. Engstrom, MD, has been named vice-president of Cancer Control and Continuing Education at the Fox Chase Cancer Center in Philadelphia, USA.

Paul Beeson MD, **Charles K. Donegan MD**, **Adrian Ostfeld MD**, **James Neel MD**, and **Dr Martha Storandt** have been appointed as new members of the National Advisory Council on Aging, USA.

Short Courses

Autumn 1984, Continuing Engineering Education Program organised by the George Washington University; London, Copenhagen, Dusseldorf, Frankfurt (Mr Michael Keenan, Manager, The George Washington University Continuing Engineering Education Program, 18 St. George's Street, Hanover Square, Mayfair, London W1R 9DE, UK).

18-20 September, **Introduction to Reinforced Plastics**, (Short Course Office (IFRP), Department of Mechanical Engineering, University of Sheffield, Mappin Street, Sheffield S1 3JD, UK).

24-27 September, **Programming in Pascal** and 23-25 October, **Control of Mechanical Systems** (The Registrar, The University of Manchester Institute of Science and Technology, PO Box 88, Manchester M60 1QD, UK).

17-19 October, **Basic High Performance Liquid Chromatography Course**, (Chrom-pack UK Ltd., Unit 4, Indecon Court, Millharbour, London E14 9TN).

19-22 November, **Training Course in Fluid Rheology** (Mr L.J. Viney, Warren Spring Laboratory, Gunnels Wood Road, Stevenage, Hertfordshire SG1 2BX, UK).

How valuable is the student grant?

from Richard Pearson

The student grant in the United Kingdom has been severely devalued by the effects of inflation. But individual circumstances vary widely.

ALTHOUGH graduate employment prospects are improving rapidly from the doldrums of the early 1980s, many will still not find an easy or speedy transition from higher education into employment (*Nature* 310, 260; 1984). With the government and its critics continuing to debate the likely level of future demand for places in the light of the declining numbers of 18-year-olds in the population, and with the apparent falling "rate of return" to studying many subjects in higher education, how attractive is the student grant in terms of supporting three years' investment in higher education?

In order to encourage student interest in subjects in demand by employers, the UK Department of Education and Science has announced that the amount undergraduates can receive from a sponsoring employer before their grant is affected will rise to £1,200 together with a £400 earnings allowance. For the lucky few who can combine sponsorship (see *Nature* 307, 488; 1984) with a full grant, this means a student income of nearly £3,500 for the academic year, double the basic grant. A recent survey for the National Union of Students (*Undergraduate Income and Expenditure*, NUS, 1984) has, however, shown that for most students half this figure may be unattainable, with many parents not making up their means-tested contribution. Although the basic grant in 1982-83 was £1,595, the average student received only £1,167, and there has been growing concern about the adequacy of this grant whose value has fallen behind the inflation rate over the past few years.

In the United Kingdom, grants are payable to the majority of students on a means-tested basis; a student living away from home at a college outside London in 1982-83 could have received a maximum grant of £1,595 plus tuition fees from his local education authority. Various supplements are also payable, for example, to those studying in London, or on a course that is longer than normal, while mature students receive a full grant with no means test. One in eight students, however, received only the minimum grant payable of £410, some because they, or their parents, refused to take the means test, while a further 5 per cent received less than £600 because their parental income was too high. Half received under £1,200 and only one in four students received the maximum grant. The total amount saved by the means test was estimated to be over £150 million in 1982-83 compared with a total

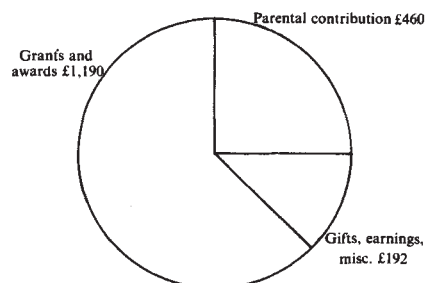


Fig. 1 Sources of student income for UK university and polytechnic students in 1982-83. (Source: NUS survey).

public expenditure on grants of about £500 million. Just over half the parents paid their assessed contribution, a rather higher proportion than in 1974-75 when only just over a quarter did so. Interestingly almost as many parents paid more than the assessed contribution as paid less than the total due and the size of the sum due from parents did not seem to affect the likelihood of the student receiving more or less than that assessed. In terms of basic grant, science and social science students got slightly higher amounts than the engineers and arts students. Average income for the academic year, including additions such as gifts and term-time earnings was £1,842, two thirds coming from the basic grant (Fig. 1).

A significant additional source of student income, outside term time, was vacation jobs, which gave an average income of £251 per student, with 10 per cent earning in excess of £700, and a few earning over £200 per week, although nearly half had no vacation job. By contrast ten years earlier 80 per cent of students had vacation jobs in the summer. Men received rather higher vacation income than women, while engineers and technology students received significantly more than the average, aided by sponsorship and enhanced prospects of vacation work. This latter factor also boosted term-time incomes of these students. Supplementary benefit and vacation grants were further sources of income out of term time for a minority of students, the average sum overall being around £100 per student.

Where then does the money go? Accommodation is the biggest outgoing. Only one in ten undergraduates in the United Kingdom lives in the parental home, while nearly half live in "official" college halls or hostels. College premises are particularly important for university students while for those at polytechnics, rented flats were the

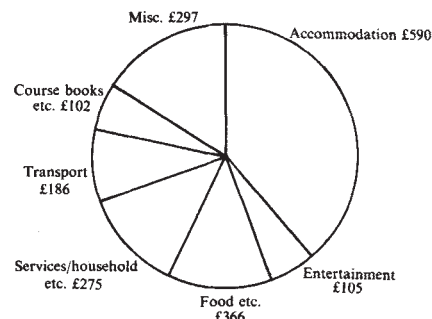


Fig. 2 Student expenditure for the academic year 1982-83. (Source: NUS survey).

most common form of accommodation. In London, more students live in their parents' home because of easier travel conditions and larger college catchment areas and the lower availability of halls of residence. The average cost of college accommodation was £24 per week, with the polytechnics cheaper than the universities, most college accommodation including some food and services. Those in private rented accommodation paid an average of £14 per week each for rent alone.

Other living costs included food and household expenses, travel and recreational. Course-related expenditure included books and course equipment; average annual expenditure was to be around £100 with university students spending most (Fig. 2) and 10 per cent apparently spending nothing at all on books. On a subject basis, medical students spent most on course equipment with social science and language students also spending above the average.

The study showed that the majority of students relied on parental support or supplementary income to support part of their studies. While for the "average" student such supplementary income was usually forthcoming, some receiving more than the notionally required sum, many had to restrict expenditure significantly to sustain themselves. The survey, by revealing the many sources of income and grants available and the various patterns of expenditure, highlights the complexities of an informed debate about student finance. Perhaps the one clear message that comes through is that to plan grants, loans and finance around a notional average case will lead to very different outcomes for different individuals. Student finance is not an exact science. □

Richard Pearson is at the Institute of Manpower Studies, Mantell Building, University of Sussex, Brighton BN1 9RF, UK.